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THE LOCATION OF EIGHTEEN GENES
IN *LEBISTES RETICULATUS*

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(With Thre ̧ Colour Plates.)

CONTENTS.

1. INTRODUCTION.

IN three earlier papers (1922 *a*, 1922 *b*, 1923 *a*) I have described certain cytological features, and some genetic researches, connected with the little viviparous tropical cyprinodont, *Lebistes reticulatus*, of which the first results were published in 1920 by Johs. Schmidt.

In these papers it was shown that there existed a series of genes, each of which determined its own particular colour pattern, in the male *Lebistes* individuals, and which were transmitted in a manner hitherto unknown, namely, from father to all sons, generation after generation, but never under any circumstances inherited by the female offspring. The genes in question were: *iridescens, maculatus, ruber* and *ferrugineus*.

To this may be added the gene mentioned only in Schmidt's work, which marks the race from the "Aquarium Society," and which I have termed *oculatus*.

It was further shown that the males were heterogametic, the females homogametic, which was explained in connection with the sex-linked inheritance of the *sulphureus* and *elongatus* genes.

The parallel cytological investigation had shown that the diploid chromosome number in both sexes was 46, the males having $44 + X + Y$, the females $44 + X + X$. There was, however, no morphological difference discernible between X and Y.

Finally, crossing-over was shown to exist, in the male *Lebistes*, between the X and the Y chromosomes, the *elongatus* gene changing its position fairly frequently, while the *sulphureus* was also found to be capable of crossing-over. The latter, however, was plainly not a single gene, but of more complex nature, as, in the only instance of crossing-over observed up to that time, only a part of the *sulphureus* characters disappeared. A further investigation of this point is dealt with in section 3 of the present work.

The result of the *Lebistes* investigations was of particular interest in that a new mode of inheritance was shown to exist, which I have termed one-sided masculine, viz. the inheritance of genes belonging to the Y chromosome. The crossing-over between the X and Y chromosomes again, which was here precisely to be expected owing to the morphological agreement between the two different types of sex chromosomes, was likewise of interest, inasmuch as this showed that the difference between the X and Y chromosomes consisted only in the fact that the Y chromosome carries a dominant male factor, whereas the X chromosome may either be said to have an allelomorphic, recessive female factor—or to have no allelomorphic factor at all. This point, to which I drew attention both in 1922 and 1923, appears to have been generally overlooked by other investigators; I therefore propose to recapitulate briefly what the experimental results have shown.

A gene, *elongatus* (*el*), involving elongation and colouring of the caudal fin in *Lebistes* males, was found (*loc. cit.* 1923 *a*) to be inherited in the typically sex-linked manner; the gene must therefore have been situated in the X chromosome. One of the male individuals has the formula $X_{el}Y_{ma}$, indicating that the *elongatus* gene, *el*, was found in the X chromosome, while the *maculatus* gene, *ma*, which *inter alia* gives a black spot on the dorsal fin (hence the name), was found in the Y chromosome. This type is shown in the accompanying Plate II,

fig. 42. Crossing with an X_oX_o female, *i.e.* one possessing no colouring factors, produced, as was to be expected, sons of the formula X_oY_{ma}, the type shown in Plate I, fig. 18, and daughters of the formula X_oX_{el}. There was, however, among a total of 73 sons, a single male individual with elongated tail, and this, on subsequent analysis, was found to be an $X_oY_{ma,\,el}$ specimen which, in contrast to the original male, transmitted the *el* gene to all its male offspring—save where renewed crossing-over took place. This in itself is enough to show that a dominant male factor is present in the Y chromosome; indeed, that the difference between an X and a Y chromosome is merely that Y possesses a dominant male-determining gene, which X lacks. For since an interchange of genes takes place on crossing-over, their constituents must be continually mingling; that they nevertheless continue to be either X or Y chromosomes can only be due to their differing essentially in nothing but the one point, a single gene. The Y chromosome in *Lebistes* differs, in principle, from the X chromosome only in possessing a male-determining Y gene, which must be regarded in the same way as any other factor. It is a matter of judgment whether we assume the presence in the X chromosome of an allelomorphic, recessive female factor, or, holding to the presence-absence theory, deny altogether the presence of any special sex factor there.

Though the male factor in *Lebistes* exhibits features differing from that for sex determination in *Drosophila*, it is hardly possible to advance any reasonable explanation other than the foregoing. T. H. Morgan (1924, 1926) does, it is true, suggest the possibility that there might, after all, be some question of greater differences in principle between the X and Y chromosomes, in that it might be imagined that the sex chromosomes in *Lebistes* were connected with a pair of autosomes, more or less as in *Ascaris*, and that crossing-over only takes place in that portion of the compound chromosome which represents the autosome, not in that part which carries the sex-determining genes. The X complex and the Y complex would then act as a pair of allelomorphic genes. This explanation of Morgan's, however, can hardly be said to be a very likely one, since it presupposes the attachment of an autosomal portion to the sex chromosomes in *Lebistes*, which is not suggested by the cytological picture; and it also involves the assumption that crossing-over only affects this supposed autosomal portion of the sex chromosomes.

Morgan's elaborate attempt to explain away the male determining gene in the Y chromosome by putting in its place a complete Y chromosome with autosome attached, must be due to a doubt as to whether

distinct, sex-determining genes exist at all. But, we may ask, what should be sex-determining, if not genes? And if this is clear, then it is only a step from the assumption of a great number of genes, some acting in the female direction, others in the male, to the assumption of a single sex-determining gene or pair of genes epistatic in its effects.

Possibly, also, it is to some extent the general idea of genes in the Y chromosome which leads Morgan to seek for a more complicated explanation. It is only natural to endeavour to bring the facts from *Lebistes* into the same angle of view as that adopted for *Drosophila*; but it must surely seem more remarkable, and more unexpected, that the X chromosome in *Drosophila* should contain many genes and Y none than that *Lebistes* should be found to have genes both in X and Y. As a matter of fact, we do not know that *Drosophila* has no distinct sex-determining gene in the X chromosome, nor can this point be decided until the deficiency investigations have been so extended as to embrace the whole of the X chromosome. Even then, there is still the same possibility in *Drosophila* as Morgan maintains in the case of *Lebistes*, that the X chromosome is only rudimentary, attached to an autosome, so that all crossings-over occurring in the X chromosome of *Drosophila* have not really taken place in the X itself, but only in the autosome, to which a sacred and inviolable X is attached.

As regards the allelomorphs of the *Lebistes* genes, which Morgan (*loc. cit.* 1926) also takes as a subject for discussion, we would refer to p. 33.

That a dominant male-determining factor in the Y chromosome of *Lebistes* should seem remarkable to us is, then, really only due to the fact that we find essentially different conditions in the well-investigated *Drosophila*, which has neither morphologically differentiating genes nor special sex-determining genes in the Y chromosome. As, however, it is common to find sometimes more, sometimes fewer genes acting upon one and the same character, it is by no means inconceivable in principle that in *Lebistes* it should be mainly a single gene which is here the male determinant. As we know, the genetics of *Lebistes* are different also in other ways from those of *Drosophila*, where we have neither Y-linked genes nor indeed any crossing-over in male individuals.

For the rest, it is already clear that in the case of *Lebistes* the same rule applies as for other organisms, both plants and animals, in which the sexes are distinct, viz. that male and female dispositions also occur in the autosomes, so that the sex chromosomes, as I have earlier (1923 *b*, p. 17) put it in the case of dioecious plants, are only to be regarded as

the normal regulating mechanism which generally deals with the determination of sex. Some observations among my *Lebistes* material show this, as will be further pointed out in the following.

In *Lebistes*, as we know, the normal thing is for the males only to have any colouring, the females being of a greyish tone throughout. Even females which are homozygous in respect of a disposition to colouring, do not show this directly, but only in the fact that their male offspring inherit the character in question. In exceptional cases, however, it has been found that old females with genes for colouring can show this phenotypically, and can also, as regards the sex organs themselves, be altered in the male direction.

Among my cultures, for instance, there were four females, born in 1923, all of which in December, 1925, became coloured, the anal fin being then also in process of transformation to an organ of copulation (*gonopodium*) otherwise characteristic of the adult male. Two of these females are shown in Plate I, figs. 15, 16, the former exhibiting a distinct transformation of the anal fin. It was remarkable that *all* the four females comprising this culture had these male sex characters more or less developed. The animals had never been paired. Their formula was known, from their ancestry, to be X_oX_{su}, that is to say, they had in the one X chromosome a disposition to *sulphureus*, which now (see section 3) is shown to be a gene complex, consisting of *coccineus* + *vitellinus*, in the other X chromosome no colour disposition at all. With regard to the effect of the *su* gene in males, I gave this (1922 *b*, p. 149) as follows:

1. Sulphur-yellow colour in the dorsal fin and a dark speck that only at times is visible in the said fin.
2. Sulphur-yellow colour in the tail and in the caudal fin.
3. Red colour in the lower edge of the caudal fin.

It will be noticed that, of these characters, the first is less distinct[1], though visible, while the two others, and especially the red on the lower edge of the caudal fin, are very conspicuous. It is interesting to note that in the transformation of these females towards the male side, a further effect of the *su* gene is revealed, viz. the occurrence of a red side spot on the tail. That I had not before noticed this effect of the *su* gene in the crossing experiments is simply due to the fact that previously it was only by observation of the males that specific effects of the genes could be discerned, and as the males in those of my cultures into which the *su* gene had hitherto been introduced always possessed

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[1] One of the females, ♀ 942, which died on December 10th, 1926, showed a very pronounced yellow colour in the dorsal fin when preserved in formalin after death.

a Y chromosome *also* containing genes involving red spots on the side, it was not easy in such cases to see that the *sulphureus* gene of the X chromosome led to the formation of the red lateral spot. Further, it is now clear that a black side speck in the tail at the base of the caudal fin must be due to the *sulphureus* gene, as *sulphureus* individuals are generally provided with such a speck; it is not visible, however, in the hermaphroditic females.

A hermaphroditic female of this kind has already been observed by Blacher (1926), curiously enough, of the same formula as the four females above mentioned; it contained the *su* gene, and its appearance was also, from Blacher's Fig. 7, similar to theirs. Blacher demonstrated the existence of sex glands of a hermaphroditic character, and I was able to discern precisely the same in one of my specimens which I sacrificed for the purpose of investigation. Nevertheless, the animals were able to copulate successfully with normal males. Two of the animals gave birth to normal offspring after pairing with $X_o Y_{ma}$ males. One of them, ♀ 942, has given birth to 82 young, of which, however, up to date only 8 $X_{su} Y_{ma}$ ♂♂ + 7 $X_o Y_{ma}$ ♂♂ can be distinguished; the other female, ♀ 944, gave birth to 17 young, among which up to date are 3 $X_{su} Y_{ma}$ ♂♂ + 2 $X_o Y_{ma}$ ♂♂. As was to be expected, the offspring segregated in the proportion of 1 : 1.

There is also another similar case in my cultures. Among some older, unpaired females of mixed ancestry there was found a female with masculine characters and with yellow colouring in the upper part of the caudal fin (see Plate I, fig. 17). It was paired with an $X_o Y_{ma}$ male and has up to date produced 91 young, of which up to the present only 23 sons have been found determinable as to formula: 12 were $X_{el} Y_{ma}$ and 11 were $X_o Y_{ma}$ so that the hermaphroditic female in question must be $X_o X_{el}$. After pairing, the yellow colouring in the caudal fin dwindled considerably; a slight extension of the anal fin, however, was retained.

One or two other cases of the transformation of older females in the male direction have been noted, and I have no doubt but that it is merely a corresponding alteration of the sex characters, such as is well known in castrated or older individuals of several animal species. On the other hand, it is not a case of aberrant, intersexual individuals produced by non-disjunction, for, if so, not all the individuals in one culture would be intersexes, and the peculiar appearance would have been observed at an earlier stage of life. Judging by their offspring they also behave as perfectly normal females.

The principal point of interest about these hermaphroditic females

is that they enable us to determine the effect on females of the gene they are known to possess; and, what is more, they show beyond all question that female individuals, *i.e.* the homogamous sex, in *Lebistes* also contain male dispositions.

Despite the fact that sex determination in *Lebistes* is of a different sort from that in *Drosophila*, where no dominant male gene is found in the Y chromosome, the difference in principle is not so great as one might at first be tempted to suppose. In *Lebistes*, also, the autosomes contain dispositions towards both male and female sex characters; the presence or absence of the Y gene is only that which *normally* regulates the sex determination.

During the past years, I have made a genetic analysis of several *Lebistes* types with a view to ascertaining whether, as my previous investigations might suggest, all the colour markings in *Lebistes reticulatus* are due to genes associated with the sex chromosomes. As already mentioned, five genes were hitherto located in the Y chromosome, and two in the X, both of which at times showed crossing-over to Y, whereas not a single gene was located in any one of the 22 autosomes.

The material on which these further investigations is based was procured partly from private owners of aquaria in Copenhagen, partly from the former Danish West Indies. With regard to the last-named material, the following should be noted. *Lebistes* is extensively used as a means to the suppression of mosquitoes, and has been introduced for this purpose into Panama, the Antilles, and elsewhere. It is placed in the water tanks of private residences, where rain water is collected in the wet seasons for use in the dry; the fish then live largely on the mosquito larvae which are constantly being deposited in the tanks.

When the Danish expeditionary vessel *Dana* came home, in July, 1922, from a cruise in the Atlantic, after visiting, *inter alia*, the former Danish West Indian Islands, the leader of the expedition, Prof. Johs. Schmidt, who is Head of the Carlsberg Laboratory, brought me some *Lebistes* which had been procured from water tanks. I take this opportunity of expressing my best thanks to Prof. Schmidt for this material. As was to be expected, these specimens contained other colour factors than those I had previously had an opportunity of analysing in fishes procured from aquarium dealers in Denmark and adjacent countries. Altogether, I have been confirmed in my previously expressed supposition, that there are in *Lebistes* a very great number of varieties, and that we shall for some time to come have no difficulty in demonstrating

the existence of genes not yet analysed, though, on the other hand, certain genes involving particularly handsome colouring or particularly decorative prolongations of the fins, may very possibly be found most widely distributed among those races which have been more especially adopted as favourites in aquaria.

2. DESCRIPTION OF NINE NEW GENES.

I shall in the following describe nine new genes, in regard to which nothing has been published hitherto. Their specific effects are shown in the coloured Plates I–III. It is a matter of judgment how many details are to be given in the description of the effect of genes. In the following I have emphasised only the most significant characters. To three of the genes formerly described have been added some minor characters to the description, as mentioned in section 3 (the *sulphureus-*complex) and section 6 (*ferrugineus, iridescens*). Owing to the increasing number of genes, these are here, in contrast to previous works, indicated by two letters representing an abbreviation of the full name. It should also be noted that the X_0X_0 females used as reaction specimens in the following experiments have throughout been taken from the *maculatus* race or the *iridescens* race, in which the females always lack disposition to colouring.

TIGRINUS (TI) AND *LUTEUS (LU)* IN X, WITH *AUREUS (AU)* IN Y.

The individual effects of these three genes in males are as follows:

tigrinus (ti) in X (see Plate III, fig. 67).

   Vertical bar pattern on tail, viz. 1–4, generally only 1–2 black pigment stripes, which are more or less conspicuous under more or less favourable conditions.

luteus (lu) in X (see Plate III, fig. 68).

   Large, early developed sulphur- or egg-coloured caudal fin portion; the caudal fin itself black-fringed.

aureus (au) in Y (see Plate III, fig. 52).

   1. 2–4 horizontally extended red spots on the side.
   2. Black spot on the breast.
   3. Black speck at root of tail or in tail fin, sometimes high up, sometimes lower down.
   4. Bright yellow colouring in the lower, but more especially in the upper part of caudal fin, which is often prolonged and fringed with black. The yellow colouring spread with increasing age, so that the two yellow patches are almost united into one.

In April, 1922, I received from a dealer named Nielsen, Gammel Kongevej 80, Copenhagen, some male *Lebistes* which were characterised

especially by a large patch of bright yellow colouring over the caudal fin, as shown in Plate I, fig. 22. Some had, moreover, distinct transverse stripes, as shown in Plate II, fig. 40.

Some of these males were paired with *Lebistes* females having no disposition to colouring, *i.e.* X_oX_o females, in order to analyse the males.

A striped male (Plate II, fig. 40), which was given No. 440, was found to possess colour genes both in the X and in the Y chromosomes. The X chromosome had indeed two colour genes, which were thus coupled, viz. *tigrinus* (*ti*), which gives the vertical striping of the tail seen in ♂ 440, and a gene, *luteus* (*lu*), which produces in the males, while still young, a bright yellow colouring of almost the whole caudal fin. These two genes however are so closely coupled that they act like a single gene-complex (see Plate III, fig. 66) which is only occasionally broken by crossing-over. Up to now, I have only observed one case of crossing-over, and here, it was the *tigrinus* gene alone which had entered into an X chromosome, while the *luteus* gene must have crossed over to the Y chromosome, of which more hereafter.

In addition to the two coupled genes in the X chromosome, ♂ 440 possessed, as mentioned, also a Y gene, viz. *aureus*, which produces in young males, *inter alia*, yellow colouring of the lower and especially of the upper margin of the caudal fin, the latter being often somewhat prolonged. In older males, the *aureus* gene is more effective (cf. Plate I, figs. 22, 23), the yellow colouring spreading over almost the whole of the caudal fin. It is thus evident that when *luteus* and *aureus*, which are very much alike, are both present in *older* males, the effect of the former is masked by that of the latter; only when the *tigrinus*, which is coupled with *luteus*, is also present, can individuals with the *luteus* gene be distinguished from those lacking it.

The result of crossing ♂ 440 with X_oX_o females was as follows. The stripes disappeared in the sons, and this character must therefore be supposed to be due to the X chromosome, which passed to the daughters; and on crossing these, it was also found that they were heterozygous in regard to the gene in question, *tigrinus*, as will be seen from the accompanying table for Exp. I. The 21 sons of ♂ 440, on the other hand, exhibited most of the other peculiarities of this type (Plate I, fig. 22) and were very much like one another. Their colouring is due solely to that gene in the Y chromosome which I have termed *aureus*.

The F_1 females were analysed by crossing with the well-known *maculatus* males whose formula is X_oY_{ma}, that is, they contain only one colour gene in the Y chromosome. This gave two types in equal

EXPERIMENT I.

Y-linked inheritance of the *aureus* (*au*) gene; X-linked inheritance
of *tigrinus* (*ti*) and *luteus* (*lu*).

Genera-tion	Parents	Female offspring	Male offspring	
F_1	♀439 × ♂440 [$X_oX_o × X_{ti,lu}Y_{au}$]	theor.: all $X_oX_{ti,lu}$ [*applied*: ♀♀528, 530, 532, 684, 686 and 690]	21 X_oY_{au} [*applied*: ♂♂593 and 691]	
back-crosses	♀♀$X_oX_{ti,lu} × ♂♂X_oY_{ma}$	theor.: $1X_oX_o : 1X_{ti,lu}X_o$	X_oY_{ma}	$X_{ti,lu}Y_{ma}$
back-crosses	♀528 × ♂529 ♀530 × ♂531 ♀532 × ♂533 ♀684 × ♂685	[*applied*: ♀♀818, 896, 898 and 900]	2 1 3 5 [*applied*: ♂♂773, 821, 841,895, 897 and 899]	0 0 2 13
back-crosses	♀686 × ♂687		13 [*applied*: ♂♂774 and 901]	8
back-crosses	Total Calculated		24 [23·5]	23 [23·5]
$F_1 × F_1$	♀592 (X_oX_o) × ♂593 (X_oY_{au})	theor.: all X_oX_o	2X_oY_{au}	
$F_1 × F_1$	♀690 × ♂691 [c/o ♀$X_oX_{ti} × X_oY_{au}$]	theor.: $1X_oX_o : 1X_{ti}X_o$	$9X_oY_{au} + 6X_{ti}Y_{au}$ calcul.: [7·5] [7·5]	
later generations	♀♀$X_oX_o × ♂♂X_{ti,lu}Y_{ma}$	theor.: all $X_oX_{ti,lu}$	X_oY_{ma} (all)	
later generations	♀772 × ♂773 ♀853 × ♂821	[*applied*: ♀894]	10 33	
later generations	Total		43 (all X_oY_{ma})	
later generations	♀♀$X_oX_{ti,lu} × ♂♂X_{ti,lu}Y_{ma}$	theor.: $1X_oX_{ti,lu} : 1X_{ti,lu}X_{ti,lu}$	X_oY_{ma}	$X_{ti,lu}Y_{ma}$
later generations	♀818 × {♂774 ♂841 ♀894 × ♂895 ♀896 × ♂897 ♀898 × ♀899 ♀900 × ♂901		3 13 12 24 10 7	1 13 12 16 10* 6
later generations	Total Calculated		69 [63·5]	58 [63·5]

The figures in heavy type indicate the individuals to be analysed.

* And 1 c/o male with *ti* and *ma* only, not further analysed.

numbers, viz. as the table shows, a total of 24 $X_o Y_{ma} + 23 X_{ti, lu} Y_{ma}$. The former type is seen in Plate I, fig. 18, the latter in Plate II, fig. 41.

It will be clearly seen from Exp. I that the transverse striping, the *tigrinus* gene and the *luteus* gene coupled with it, are inherited in sex-linked fashion as a gene complex; the experiment also places it beyond doubt that the *aureus* gene is associated with the Y chromosome. It will also be noted that an F_1 male, ♂ 593, transmitted the *aureus* gene to both its sons.

On crossing two F_1 individuals with one another, ♀ 690 and ♂ 691 (see Exp. I), we obtained, as was to be expected, a segregation. We should have expected to find ♀ 690 with two genes in one X chromosome, viz. of the formula $X_o X_{ti, lu}$; but the result of the cross shows that the *luteus* gene was not found in this female, which must have arisen from a crossing-over at the reduction division of the father, ♂ 440, viz. by fertilisation of an X_o egg with an X_{ti} spermatozoon. For not a single one of the 15 sons of ♀ 690 had the caudal fin so highly coloured as would have been the case had the *luteus* gene been present, whereas naturally all 15 had yellow especially in the upper part of the caudal fin, all having Y_{au}. Segregation could thus only be discerned in regard to the *tigrinus* gene, the two types appearing as shown in Plate I, fig. 23, and Plate II, fig. 37.

The combined sex-linked inheritance of the *tigrinus* gene and one-sided masculine inheritance of the *au* gene explains the segregation above noted in the F_2 generation. As all the males showed the Y_{au} gene, we have here also proof that it was not a simple case of dominance, when the 21 F_1 males proved to be of the *aureus* type, but due to the fact that the gene in question is located in the Y chromosome, and is thus constantly transmitted from father to all sons.

Exp. I further shows the results of crossings with four segregated females, ♀♀ 818, 896, 898 and 900, which can, theoretically, be either $X_o X_{ti, lu}$ or $X_o X_o$. As will be seen, they proved to be all four of the first-named formula, half their sons having the formula $X_o Y_{ma}$ and half $X_{ti, lu} Y_{ma}$; there were in all 57 and 46 respectively of the two types. ♀ 894, which, as will be seen, should theoretically be $X_o X_{ti, lu}$ gave, as was to be expected, offspring which segregated in the same way.

Finally, Exp. I shows that two segregated $X_{ti, lu} Y_{ma}$ males, ♂♂ 773 and 821, when crossed with $X_o X_o$ females, gave only $X_o Y_{ma}$ sons, in all 43 *maculatus* males.

As mentioned, only a few of the males received were striped. Most

<center>Experiment II.</center>

<center>Y-linked inheritance of the *aureus (au)* gene.</center>

Parents	Female offspring	Male offspring
♀♀X_oX_o × ♂♂X_oY_{au}	theor.: all X_oX_o	all X_oY_{au}
♀ 441 × ♂ **442**		5
♀ 594 × ♂ **595**		5
♀ 600 × ♂ **601**		2
Total		12 (all X_oY_{au})

had evidently only the *aureus* gene. This is also apparent from the crosses in which three of the original males were used (see Exp. II).

The 12 sons were, as was theoretically to be expected, all of the same appearance as the fathers.

Other results besides those mentioned in Exp. I have also shown that the sex-linked *tigrinus* gene can occur independently of the *luteus* gene. This is shown by some crosses with ♂ 603 $X_{ti}Y_{au}$, which was procured from the same source as the male *Lebistes* above mentioned. It was of the same appearance as the males used in Exp. II, but had presumably also the *tigrinus* gene in its X chromosome. It should be noted that it was not directly observed that ♂ 603 was striped on the tail like ♂ 440, but as the crossing analysis shows inheritance of the disposition to this, and as a note I made some time back (April, 1923) while the animal was alive can almost certainly be taken as implying this; and as, moreover, the stripes may be difficult to see, especially when the animals are young, or in poor condition, there is no doubt but that ♂ 603's formula must have been $X_{ti}Y_{au}$. Exp. III shows the result of crossing with this male.

None of the six sons exhibited stripes, but on crossing a daughter, ♀ 739, which of course should have had the formula X_oX_{ti} with a *maculatus* male, X_oY_{ma}, we obtained, as the table shows, a very distinct segregation 1 : 1, viz. 29 X_oY_{ma} + 30 $X_{ti}Y_{ma}$. The two types are shown in Plate I, fig. 18, and Plate II, fig. 38. The striping is thus sex-linked in its inheritance. This is further confirmed by the behaviour of the three males 875, 877 and 879 of the formula $X_{ti}Y_{ma}$, derived from the last-mentioned cross. When paired with X_oX_o females, these three males produced exclusively X_oY_{ma} sons, in all 62.

EXPERIMENT III.

X-linked inheritance of the *tigrinus* (*ti*) gene.

Genera-tion	Parents	Female offspring	Male offspring
F_1	$♀♀ X_o X_o × ♂ X_{ti} Y_{au}$	theor.: all $X_o X_{ti}$	all $X_o Y_{au}$
	$♀ 602 \atop ♀ 618$ } $× ♂ 603$	[*applied*: ♀ 739]	1 5
	Total		6 (all $X_o Y_{au}$)
back-cross	$♀ 739 × 740$ [$X_o X_{ti} × X_o Y_{ma}$]	theor.: $1 X_o X_{ti} : 1 X_o X_o$	$29 X_o Y_{ma} + 30 X_{ti} Y_{ma}$ [*applied*: ♂♂ 875, 877 and 879]
later generations	$♀♀ X_o X_o × ♂♂ X_{ti} Y_{ma}$	theor.: all $X_o X_{ti}$	all $X_o Y_{ma}$
	$♀ 874 × ♂ 875$ $♀ 876 × ♂ 877$ $♀ 878 × ♂ 879$		21* 18 23
	Total		62 (all $X_o Y_{ma}$)

* Furthermore, 2 c/o males were found with *ma* and *ti*, this last gene presumably
having crossed over to the Y chromosome.

I have also had *Lebistes* with Y_{au} from another source, viz. from
Hr. Hansen, Øresundsvej 2, Copenhagen. Even by direct observation
it seemed likely that these *Lebistes* males would have the same Y-linked
gene as those from Hr. Nielsen, Gammel Kongevej; and probably the
animals from these two distinct quarters were themselves originally
derived from one and the same source.

Three males were used for crossing, viz. ♂♂ 460, 462 and 464; they were
all entirely similar in appearance, roughly that shown in Plate I, fig. 22.
None' of the specimens had transverse stripes like ♂ 440; neither the
tigrinus nor the *luteus* gene could have been present in their X chromo-
some. This last is here empty, so that the formula will be $X_o Y_{au}$, and
thus agrees with the males analysed in Exp. II. The crossing analysis
also showed this (see Exp. IV).

As will be seen, all the 45 sons were of the same appearance as the
fathers. An F_1 male, ♂ 586, was crossed again with an $X_o X_o$ female,
and yielded, as was to be expected, again males of the same appearance,
viz. 4 $X_o Y_{au}$.

The F_1 females would be expected to have the formula $X_o X_o$. Two
of these were paired, as will be seen, with *maculatus* males, $X_o Y_{ma}$, and,

Experiment IV.

Y-linked inheritance of the *aureus (au)* gene. (The origin of these three males is different from that of the males in Exp. II.)

Genera-tion	Parents	Female offspring	Male offspring
F_1	$♀♀ X_0 X_0 \times ♂♂ X_0 Y_{au}$	theor.: all $X_0 X_0$	all $X_0 Y_{au}$
	$\begin{array}{l}♀459\\♀519\\♀551\\♀582\end{array}\Big\} \times ♂ 460$	[*applied*: ♀587 here, ♀762 in Exp. X]	$\begin{array}{l}6\\7\\4\\3\end{array}$
	$\begin{array}{l}♀461\\♀473\\♀524\end{array}\Big\} \times ♂ 462$		$\begin{array}{l}5\\2\\11\end{array}$
	$♀463 \times ♂ 464$	[*applied*: ♀750]	7 [*applied*: ♂ 586]
	Total		45 (all $X_0 Y_{au}$)
back-crosses	$♀585 \times ♂ 586$ [$X_0 X_0 \times F_1 ♂ X_0 Y_{au}$]		$4 X_0 Y_{au}$
	$F_1 ♀♀ X_0 X_0 \times ♂♂ X_0 Y_{ma}$	theor.: all $X_0 X_0$	all $X_0 Y_{ma}$
	$\begin{array}{l}♀587 \times ♂ 588\\♀750 \times ♂ 680\end{array}$		$\begin{array}{l}3\\12\end{array}$
	Total		15 (all $X_0 Y_{ma}$)

as expected, transmitted no colouring gene to their sons, their X chromosomes being empty. All the 15 sons were of the *maculatus* type.

It will be seen that the three new colour genes here described in *Lebistes* are all located in the sex chromosomes, viz. two of them in the X and one in the Y. Only in the case of the third, *luteus*, has it been shown that it can proceed from the X chromosome, presumably of course by crossing-over, to Y. As to the frequency of such crossing-over, we can at present only say that it seems to be relatively rare.

Zebrinus (ze) in Autosome 1.

In January, 1918, I selected from among some *Lebistes* received from Hamburg a male specimen distinguished *inter alia* by vertical stripes on the tail, as if it possessed the previously mentioned *tigrinus* gene, though this, as a matter of fact, was not known to me until later, and is actually of a different origin. In contradistinction to the *tigrinus* gene, which was associated with the X chromosome, the *zebrinus* is

situated in an autosome which we will call No. 1 of the 22 autosomes found in *Lebistes*.

zebrinus (*ze*) in autosome 1 (see Plate III, fig. 71).

A barred pattern of vertical stripes on the tail, viz. 2–5, generally 3 dark pigment stripes. The effect resembles that of the *tigrinus* gene, but is as a rule more pronounced.

I have been making investigations for eight years with this gene, though it is only during the last few years that they have been systematic and thorough; that is, after I had perceived that this gene differed as regards its mode of inheritance from the others known to me in *Lebistes*. The inheritance is as that of an ordinary mendelian dominant factor.

That I have not previously published anything regarding this gene is due to the fact that sex-linked inheritance with frequent crossing-over between the X and the Y chromosome might easily be confused with ordinary mendelian inheritance, and it was therefore desirable to have an abundance of numerical material. There can now no longer be any doubt but that this secondary sex character, in contrast to those previously known in *Lebistes*, is inherited through an autosome.

The proof of autosomal inheritance lies partly in the fact that certain males have proved homozygous with regard to the *zebrinus* gene, producing exclusively transverse barred sons, while others only transmit *zebrinus* to half their sons; partly in the fact that the barred *Lebistes* male can transmit the *ze* gene equally well to sons and daughters.

These facts might, however, still be explained by sex-linked inheritance, if the *Lebistes* males were homogametic and the females heterogametic; but, as I have long since shown, the reverse is the case. And as, moreover, *Lebistes* females with the *ze* gene are often heterozygous and transmit the disposition to half their sons, the position is perfectly clear.

The original *zebrinus* male, ♂ 171, was evidently homozygously barred, for on crossing with an $X_o X_o$ female, ♀ 197, it produced, as far as could be seen from the offspring preserved in formalin, exclusively barred sons, viz. 20 in all, while when crossed with three other females, likewise without colour disposition, he produced 4, 1 and 12 sons respectively, all, as far as could be ascertained, transversely barred. A like result would, of course, be produced if the Y chromosome contained the *zebrinus* gene, since all the sons derive the Y chromosome from their father. As will be shown in the following, however, the females also receive the *ze* gene.

EXPERIMENT V.

Autosomal inheritance of the *zebrinus* (ze) gene.   ♂ **369** appears to be homozygous for *zebrinus* (formula: $1_{ze}1_{ze}X_oY_{ma}$).

Generation	Parents	Female offspring	Male offspring	
F_1	♀367 × ♂ **369** $[1_o1_oX_oX_o × 1_{ze}1_{ze}X_oY_{ma}]$	theor.: all $1_o1_{ze}X_oX_o$ [*applied*: ♀♀ 474, 476, 480, 482, 709 and 711]	19 $1_o1_{ze}X_oY_{ma}$ [*applied*: ♂♂ 489, 491, 493, 710, 712, 718, 720 and 722]	
back-crosses of F_1 to the recessive type	♀♀ $1_o1_{ze}* × ♂♂ 1_o1_o$	theor: $1\ 1_o1_o : 1\ 1_{ze}1_o$	$1_{ze}1_o$	1_o1_o
	♀474 × ♂ 475 ♀476 × ♂ 477 ♀480 × ♂ 481 ♀482 × ♂ 483		4 2 9 9	5 0 10 10
	Total Calculated		24 [24·5]	25 [24·5]
	♀♀ $1_o1_o × ♂♂ 1_o1_{ze}$:	theor.: $1\ 1_o1_o : 1\ 1_{ze}1_o$	$1_{ze}1_o$	1_o1_o
	♀488 × ♂ 489 ♀490 × ♂ 491 ♀492 × ♂ 493 ♀717 × { ♂ 710 / ♂ 718 ♀719 × ♂ 720 ♀721 × ♂ 722		1 2 3 1 7 1 14	4 2 2 4 8 2 13
	Total Calculated		29 [32·0]	35 [32·0]
$F_1 × F_1$	♀♀ $1_o1_{ze} × ♂♂ 1_o1_{ze}$:	theor.: $1\ 1_o1_o : 2\ 1_{ze}1_o : 1\ 1_{ze}1_{ze}$	$1_{ze}1_{ze} + 1_{ze}1_o$	1_o1_o
	♀709 × ♂ 710 ♀711 × ♂ 712	[*applied*: ♀823]	3 20 [*applied*: ♂831]	0 4
	Total Calculated		23 [20·25]	4 [6·75]
later generations	♀823 × ♂ 824 $[1_o1_{ze} × 1_o1_o]$		with *ze* 16	without *ze* 19
	♀830 × ♂ 831 $[1_o1_o × 1_{ze}1_{ze}]$		7	0

* In order to facilitate a general view, the X and Y chromosomes are omitted from the formulae, as they are of no importance in this connection. The recessive types used for back-crossing were nearly all X_oX_o in the case of the females, and X_oY_{ma} in the case of the males.

A daughter, ♀ 294, of the original, transversely barred ♂ 171, was crossed with a *maculatus* male, ♂ 295, and in this race, no transverse barred specimens had ever appeared. A barred F_2 male, No. 369 (see Plate II, fig. 46) from this crossing, formed the starting point of a long series of further pairings, as shown in Exp. V.

The 19 sons of ♂ 369 were, as will be seen, all barred, but an investigation of the next generation shows decisively that the *zebrinus* gene is not restricted to the Y chromosome, as 4 daughters and 7 sons, backcrossed to the recessive, non-barred type (♀♀: $1_o1_oX_oX_o$ and ♂♂: $1_o1_oX_oY_{ma}$) all showed 1 : 1 segregations, all being heterozygously barred, 1_o1_{ze}. I counted in all, after such back-crossings, 53 barred and 60 non-barred, as shown in the table. *Ze* must therefore be located in an autosome. That the 2 sons of ♀ 476 both had the *zebrinus* gene is of course quite accidental.

As will be seen from the next two lines of the table, the offspring of ♂ 369 were also paired one with another; *i.e.* individuals which should, in the case of both sexes, be heterozygous in respect of *ze*. Considering the small number of sons, the divergence from what should theoretically be expected, *i.e.* a proportion of 3 : 1, is not very striking; we had 23 with *ze* and 4 without.

One of the 23 males, ♂ 831, which was paired again with a 1_o1_o female, must presumably have been $1_{ze}1_{ze}$ for its 7 sons were all found to be barred. A sister to these males, ♀ 823, on the other hand, paired with a male 1_o1_o, proved to be heterozygous, 1_o1_{ze}, for 16 of its sons were barred, and 19 were not.

Several other crosses have also been made with *zebrinus* individuals, but I do not consider it necessary to give the whole of the complicated pedigree. The most interesting thing to note is, how a great number of *Lebistes* which, from their ancestry (and appearance) should, theoretically, have been heterozygous in respect of the *zebrinus* gene, behaved when back-crossed with individuals lacking that factor. Exp. VI gives the crossings of this category not included in Exp. V.

The segregation shows beyond question a 1 : 1 proportion. On addition of the back-crossing results in Exps. V and VI, which are, of course, the same in all essentials, we obtain 192 males with *zebrinus* and 181 without.

In this connection, it should be noted that the effect of the *zebrinus* gene may occasionally be observed also in females. Thus in 1922, for instance, it was noticed that two females of the same ancestry as the mentioned ♂ 369 had barred tails, the marking being visible when viewed

Experiment VI.

Autosomal inheritance of the *zebrinus* gene. Other crossings of hetero-zygous *zebrinus* individuals ($1_o 1_{ze}$) with the $1_o 1_o$ type.

Parents	Male offspring	
♀♀ $1_o 1_{ze}$ × ♂♂ $1_o 1_o$	with *ze*	without *ze*
♀ 382 × ♂ 383	2	4
♀ 655 × ♂ 656	8	2
♀ 705 × ♂ 706	2	2
♀ 707 × ♂ 708	4	1
♀♀ $1_o 1_o$ × ♂♂ $1_o 1_{ze}$:		
♀ 496 × ♂ 497	4	1
♀ 498 ⎱ × ♂ 499	4	1
♀ 525 ⎰	3	5
♀ 659 × ♂ 660	2	1
♀ 663 × ♂ 664	13	7
♀ 667 × ♂ 668	6	5
♀ 669 × ♂ 670	12	13
♀ 671 × ♂ 672	1	7
♀ 697 × ♂ 666	1	2
♀ 795 ⎱ × ♂ 797	4	6
♀ 796 ⎰	8	3
♀ 798 ⎱ × ♂ 800	4	6
♀ 799 ⎰	6	5
♀ 801 ⎱ × ♂ 803	12	7
♀ 802 ⎰	6	7
♀ 804 ⎱ × ♂ 806	16	12
♀ 805 ⎰	5	5
Total	123	102
Calculated	[112·5]	[112·5]

Experiment VII.

Two phenotypically barred females appeared to be probably homozygous $1_{ze} 1_{ze}$.

Parents	Male offspring	
	with *ze*	without *ze*
$1_{ze} 1_{ze}$ ⎰ ♀ 484 × ♂ 485 ⎱ $1_o 1_o$	4	0
⎱ ♀ 486 × ♂ 487 ⎰	5	0
Total	9	0

in a favourable light. The animals were then something over two years old. These two females, 484 and 486, were probably homozygously barred, as all their sons had the barred marking (see Exp. VII).

Three daughters of the mentioned ♀ 486 were analysed by crossing with *maculatus* males, and proved, as expected, to be heterozygous in regard to *ze*. These daughters were numbered ♀♀ 655, 705 and 707, and are shown in Exp. VI, where the segregation will be seen. In all 12 offspring of the barred females had the *zebrinus* gene, which was either demonstrated by direct observation (the sons) or by crossing-analysis (daughters); none, however, lacked *ze*. Everything thus seems to suggest that the two females, 484 and 486, were homozygous in regard to *ze*. Whether the visible barred marking in these females is actually due to their being homozygous is not certain.

ARMATUS (AR) IN Y.

In 1922, I received from Hr. Jensen, Husumgade, Copenhagen, a *Lebistes* male with a remarkably long, sword-like lower prolongation of the caudal fin as shown in Plate II, figs. 24–25. The animal in question was said to be a hybrid between *Xiphophorus Helleri* and *Lebistes reticulatus*. But the number of rays in the dorsal fin, which is twice as great in *Xiphophorus* as in *Lebistes*, at once showed that this was incorrect, for the number of rays here was 7, as generally found in *Lebistes*, and the specimen had, apart from the sword-like prolongation of the fin, no resemblance to *Xiphophorus*. Indeed, as far as my experience goes, it is impossible to cross the two species. I may mention that the chromosome number in *Xiphophorus* (male) is 23 or 24 haploid, *i.e.* identical with or very near to that of *Lebistes*, but I have not made any further investigation of the cytology of this species.

The prolongation of the caudal fin, and other characters in the mentioned male *Lebistes*, ♂ 458, were due, as genetic analysis showed, exclusively to a single gene, *armatus*, located in the Y chromosome, and with typically one-sided masculine inheritance.

armatus (ar) in Y (see Plate III, fig. 53).

1. Two to three red patches on the side, the largest low down on the tail. Often also a red spot at the root of the caudal fin itself.

2. One to two black spots on the side in front of the dorsal fin. One is always high up, and often divides the foremost red patch into two.

3. A little black speck in the caudal fin or occasionally farther back on the tail.

4. A long, dagger-shaped, sulphur-coloured prolongation of the lower edge of the caudal fin.

Experiment VIII.

Y-linked inheritance of the *armatus* (*ar*) gene.

Generation	Parents	Female offspring	Male offspring	
F_1	♀ 457 × ♂ 458 [$X_o X_o$ × $X_o Y_{ar}$]	theor.: all $X_o X_o$ [*applied*: ♀♀ 636 and 646]	5 $X_o Y_{ar}$ [*applied*: ♂ 640]	
back-crosses	♀ 644 × ♂ 640 [$X_o X_o$ × $X_o Y_{ar}$]	theor.: all $X_o X_o$ [*applied*: ♀ 749]	25 $X_o Y_{ar}$ [*applied*: ♂♂ 769 and 846]	
	♀♀ $X_o X_o$ (segregated) × ♂♂ $X_o Y_{ma}$	theor.: all $X_o X_o$	all $X_o Y_{mu}$	
	♀ 636 × ♂ 632 ♀ 646 × ♂ 698 ♀ 749 × ♂ 654*		1 3 9	
	Total		13 (all $X_o Y_{mu}$)	
later generations	♀ $X_o X_{el}$ × ♂♂ $X_o Y_{ar}$	theor.: 1 $X_o X_o$: 1 $X_o X_{el}$	$X_o Y_{ar}$	$X_{el} Y_{ar}$
	♀ 768 × { ♂ 769 ♂ 846		3† 4	2
	Total Calculated		7 [4·5]	2 [4·5]

* ♂ 654 had, however, one more gene apart from these, but no further mention need be made of this in the present connection.

† We might here expect segregation of $X_{el} Y_{ar}$ males; as it happened, however, none of the three males had X_{el}.

As will be seen from Exp. VIII above, all sons of *armatus* males inherit throughout the appearance of the father, even where *armatus* individuals are crossed for generation after generation with $X_o X_o$ females of other race. It is thus indisputable that the *armatus* gene is located in the Y chromosome.

Pauper (*pa*) and *cinnamomeus* (*ci*) in Y, with *lineatus* (*li*) in X.

Among the *Lebistes* brought home from the West Indies there was a male, ♂ 506 (see Plate II, fig. 27), which was found to have a single gene for colour marking in the Y chromosome. This gene I have called *pauper*, from its being, for a Y gene, relatively poor in decorative value. In another male, ♂ 501 (see Plate II, fig. 28), from the same locality, with handsomer colouring, it was found that two other genes besides *pauper* were present, viz. one more in the Y chromosome, *cinnamomeus*, and one in the X chromosome, called *lineatus*. The original, ♀ 536, was

also analysed, and found to contain the *lineatus* gene, though this was of course not phenotypically visible in the individual itself, but only appeared in the offspring.

The three genes, whose individual effects are here appended, are thus in two cases of one-sided masculine inheritance, the third being sex-linked as shown in the following crossing experiments.

pauper (*pa*) in Y (see Plate III, fig. 58).

    1. Small, horizontally extended red patch set high up on the hinder part of the tail; occasionally bisected by the lateral line.

    2. A black speck on the side behind the red patch.

    3. Occasionally, there may also be a black anal speck and a black spot on the breast; also a little red in the dorsal fin.

cinnamomeus (*ci*) in Y (see Plate III, fig. 59).

    1. A peculiar metallic sheen, of a warm brownish yellow, on the side of the body; most conspicuous in direct light.

    2. A minute faintly yellow flame proximally in the lower part of the caudal fin, not always visible.

The effect of the *ci* gene is on the whole more difficult to recognise than that of the other genes, and, as already mentioned, does not involve any sharply defined marking on the body.

lineatus (*li*) in X (see (Plate III, fig. 56).

    1. A red, black-edged stripe in the upper margin of the caudal fin, which is often somewhat prolonged.

    2. Generally a black speck at the root of the caudal fin.

In this case also analysis was made by crossing the original males with females of the *maculatus* race, which has no disposition to colouring ($X_o X_o$), with subsequent back-crossing to the *maculatus* race.

In the case of $\male$ 506 (see Plate II, fig. 27), the formula for which was $X_o Y_{pa}$, Exp. IX shows the analysis of this.

<div align="center">

EXPERIMENT IX.

Y-linked inheritance of the *pauper* (*pa*) gene.

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Generation	Parents	Female offspring	Male offspring
F_1	$\female$ 554 × $\male$ 506 [$X_o X_o$ × $X_o Y_{pa}$]	theor.: all $X_o X_o$ [*applied*: $\female$ 747]	4 $X_o Y_{pa}$ [*applied*: $\male\male$ 724 and 776]
back-crosses	$\female\female$ $X_o X_o$ × $\male\male$ $X_o Y_{pa}$	theor.: all $X_o X_o$	all $X_o Y_{pa}$
	$\female$ 723 × $\male$ 724 $\female$ 775 × $\male$ 776		20 8
	Total		28 (all $X_o Y_{pa}$)
	$\female$ 747 [$X_o X_o$ segregated] × $\male$ 748 [$X_o Y_{ma}$]	theor.: all $X_o X_o$	1 $X_o Y_{ma}$

As all male offspring of the original $\male$ 506, both in F_1 and after back-crossing of F_1 males, were altogether identical with this, it is evident from this experiment that we have here to deal with a Y-linked gene. This is further confirmed by the analysis of $\male$ 501, which also had the *pauper* gene, as described in the following.

The original West Indian $\male$ 501 (see Plate II, fig. 28) was of the formula $X_{li}Y_{pa,\,ci}$. A first crossing with an X_oX_o female, $\female$ 500, at once suggested that the red stripe in the upper margin of the caudal fin, *lineatus*, was associated with the X chromosome, for 12 sons all lacked the stripe, which was due to the *lineatus* gene. This last had been transmitted to all the daughters of $\male$ 501, as was proved by a cross of 4 of them (see Exp. X).

As regards the *cinnamomeus* gene, it is only in the last few generations that it became evident that this was inherited through the Y chromosome. Owing to the inconspicuous effect of the *ci* gene, it was partly overlooked in the first years. It is therefore dealt with separately here, and not mentioned in Exp. X, which gives only the inheritance of the *lineatus* and *pauper* genes.

Exp. X places it beyond doubt that the *pauper* gene is always transmitted from the father to all sons; which conclusion was, indeed, already indicated by Exp. IX. All the 97 sons noted in Exp. X as offspring of males with the *pauper* gene showed the presence of this gene. In Exp. IX and X, I investigated altogether 129 sons of 11 different *pauper* males, belonging to various generations; in no single instance did these sons lack the *pauper* gene, which is thus linked to the Y chromosome.

That the *lineatus* gene, on the other hand, is situated in the X chromosome, is no less certain, for none of the 30 sons of males with *lineatus* noted in Exp. X had received this gene from the father, whereas all the daughters of *li* males further analysed proved to be heterozygous in respect of this gene. When crossed with *maculatus* males, or $X_{li}Y_{ma}$ males, they segregated in all 59 $X_{li}Y_{ma}$ (see Plate II, fig. 29) and 72 X_oY_{ma} sons (see Plate I, fig. 18), which agrees with the proportion theoretically to be expected, viz. 1 : 1.—

We have still to consider the *cinnamomeus* gene, which formed an item in Exp. X, where the original, $\male$ 501, possessed it. As already mentioned, it was only in the later generations that it was discovered to be—like *pauper*—associated with the Y chromosome. As, however, the original *pauper* individual in Exp. IX, $\male$ 506, and all its offspring lacked *ci*, it seems as if this gene can, in crossing over, disappear from the

EXPERIMENT X.

Y-linked inheritance of the *pauper* (*pa*) gene.; X-linked inheritance of the *lineatus* (*li*) gene. (The individuals marked * are segregated from other experiments.)

Generations	Parents	Female offspring	Male offspring	
F_1	♀ 500 × ♂ 501 [$X_o X_o \times X_{li} Y_{pa}$]	theor.: all $X_o X_{li}$ [*applied*: ♀♀ 628, 699, 701 and 703]	$12 X_o Y_{pa}$ [*applied*: ♂♂ 609 and 627]	
back-crosses	♀♀ $X_o X_o \times$ ♂♂ $X_o Y_{pa}$:	theor.: all $X_o X_o$	all $X_o Y_{pa}$	
	♀ 608 × ♂ 609 ♀ 626 × ♂ 627	[*applied*: ♀ 789] [*applied*: ♀♀ 790, 801, 802, 804 and 805]	1 11 [*applied*: ♂♂ 778, 782, 791, 794 and 857]	
	Total		12 (all $X_o Y_{pa}$)	
	♀♀ $X_o X_{li} \times$ ♂♂ $X_o Y_{ma}$:	theor.: 1 $X_o X_o$: 1 $X_o X_{li}$	$X_o Y_{ma}$	$X_{li} Y_{ma}$
	♀ 628 × ♂ 529			1 [*applied*: ♂ 648]
	♀ 699 × ♂ 700 ♀ 701 × ♂ 702 ♀ 703 × ♂ 704		18 6 7	17 4 7 [*applied*: ♂♂ 903, 905 and 907]
	Total Calculated		31 [30·0]	29 [30·0]
later generations	♀ 762 (from Exp. IV) × ♂ 648 [$X_o X_o \times X_{li} Y_{ma}$]	theor.: all $X_o X_{li}$ [*applied*: ♀♀ 902, 904 and 906]	$18 X_o Y_{ma}$	
	♀♀ $X_o X_o \times$ ♂♂ $X_o Y_{pa}$:	theor.: all $X_o X_o$	all $X_o Y_{pa}$	
	♀ 777 × ♂ 778 ♀ 781 × ♂ 782 ♀ 790 × ♂ 791 ♀ 792*} × ♂ 794 ♀ 793*} ♀ 856 × ♂ 857		4 14 21 5 2 27	
	Total		73 (all $X_o Y_{pa}$)	
	♀♀ $X_o X_o$ (segregated) × ♂♂ $X_o Y_{ma}$:	theor.: all $X_o X_o$	all $X_o Y_{ma}$	
	♀ 789 × ♂ 674 ♀ 801} × ♂ 803* ♀ 802} ♀ 804} × ♂ 806* ♀ 805}		7 19 13 26 9	
	Total		74 (all $X_o Y_{ma}$)	
	♀♀ $X_o X_{li} \times$ ♂♂ $X_{li} Y_{ma}$:	theor.: 1 $X_o X_{li}$: 1 $X_{li} X_{li}$	$X_o Y_{ma}$	$X_{li} Y_{ma}$
	♀ 902 × ♂ 903 ♀ 904 × ♂ 905 ♀ 906 × ♂ 907		14 19 7	15 14 2

Y chromosome, or else it must have arisen or become eliminated by mutation. Crossing-over has not hitherto been observed with certainty, and in no case has it, up to now, been inherited through females. It was thus easy to arrive at two constant "races," the males of one having the formula X_oY_{pa} (see Plate II, fig. 27) and those of the other $X_oY_{pa,ci}$ (see Plate II, fig. 39), while the females in both are X_oX_o.

Owing to the inadequacy of the earliest observations in regard to occurrence of the *ci* gene, I give no pedigree of this here, but a brief general description.

Of 12 sons of the original, ♂ 501, 8 at any rate are known to have had *cinnamomeus*, and even one of the sons not noted as possessing the *ci* gene transmitted this to several of its sons, which shows that the observations must have been inadequate, since there is no instance on record among *Lebistes* of genes for colour pattern being recessively present. They have always been directly discernible in the males possessing them, and it is probably only because the effect of the *ci* gene is relatively weak and only observed after some experience, and when the animals are in a good state of health, that it escaped notice in several specimens. That the inheritance of *ci* is one-sided masculine becomes a certainty when we mention that the sons of the mentioned ♂ 627 used for crossing, viz. ♂♂ 778, 782, 791 and 794, have up to the present produced 30 fully grown sons, which, with the exception of a single specimen, all exhibited the presence of the *ci* gene; possibly this one may also develop the character as it grows older.—

As mentioned on p. 20, one of the original females brought home young from the West Indies also revealed the sex-linked *lineatus* gene. This female, numbered ♀ 536, was paired with a *maculatus* male, and the result of the analysis thus commenced is shown in Exp. XI.

It will be seen from Exp. XI that the West Indian female was heterozygous in regard to the *lineatus* gene, as 2 of its 3 daughters had inherited the *li* gene. Only one son attained full maturity and exhibited the effect of the *li* gene; and it was this which led to the pairing of its sisters in order to make sure as to the sex-linked inheritance of the *lineatus* gene. The solitary son left no offspring. Of the 3 daughters, ♀ 717 evidently did not possess *li*, for none of the 15 + 5 sons inherited it. Both the other daughters, however, ♀ 719 and 721, had the formula X_oX_{li} since, when crossed with *maculatus* males, they produced sons of both kinds, some $X_{li}Y_{ma}$ (12 spec.) and some X_oY_{ma} (18 spec.). As was to be expected, the male offspring of one of these $X_{li}Y_{ma}$ males,

EXPERIMENT XI.

The original West Indian ♀ **536** contains the X-linked *lineatus* (*li*) gene. (The individuals marked * are segregated from other experiments.)

Genera-tion	Parents	Female offspring	Male offspring	
F_1	♀ 536 × ♂ 537* [$X_o X_{li}$ × $X_o Y_{ma}$]	theor.: $1 X_o X_o : 1 X_o X_{li}$ [*applied*: ♀♀ 717, 719 and 721]	$1 X_{li} Y_{ma}$	
back-crosses	♀ $X_o X_o$ (segregated) × ♂♂ $X_o Y_{ma}$	theor.: all $X_o X_o$	all $X_o Y_{ma}$	
	♀ 717 × $\begin{cases} ♂ 718* \\ ♂ 710* \end{cases}$		15 5	
	Total		20 (all $X_o Y_{ma}$)	
	♀♀ $X_o X_{li}$ × ♂♂ $X_o Y_{ma}$	theor.: $1 X_o X_o : 1 X_o X_{li}$	$X_o Y_{ma}$	$X_{li} Y_{ma}$
	♀ 719 × ♂ 720* ♀ 721 × ♂ 722*		2 16	1 11 [*applied*: ♂797]
	Total Calculated		18 [15·0]	12 [15·0]
later genera-tions	♀ 795* × ♂ 797 [$X_o X_o$ × $X_{li} Y_{au}$]	theor.: all $X_o X_{li}$	10 $X_o Y_{ma}$	

♂ 797, paired with an $X_o X_o$ female, were all $X_o Y_{ma}$ (10 in all), thus confirming the sex-linked mode of inheritance of this gene.

The *maculatus* male, ♂ 782, with which ♀ 721 $X_o X_{li}$ was paired, was barred, and proved to be heterozygous for the *zebrinus* gene. Consequently, three genes must have been implicated in this crossing, viz. *lineatus*, *zebrinus* and *maculatus*, the inheritance of which is sex-linked, autosomal and one-sided masculine respectively. The following arrangement shows the segregation as a whole, only the sons being here considered.

$$♀ 721 \qquad × \qquad ♂ 722$$
$$1_o 1_o X_o X_{li} \qquad\qquad 1_o 1_{ze} X_o Y_{ma}$$

	$1_o 1_o X_o Y_{ma}$		$1_o 1_o X_{li} Y_{ma}$		$1_o 1_{ze} X_o Y_{ma}$		$1_o 1_{ze} X_{li} Y_{ma}$
Theoretic	1	:	1	:	1	:	1
Actual	10		4		6		7

Both the parents and 12 of the sons are shown in Plate I, figs. 1–14. The four types, which should theoretically appear in equal numbers,

will be easily distinguished. The barred pattern *ze*, or the lack of it, is freely combined with the red stripe in the caudal fin, *li*, or the lack of it. All, of course, exhibit the *maculatus* characters.

It is thus evident that the West Indian *Lebistes* female was heterozygous for the *lineatus* gene, which is not surprising, since it was presumably related to the West Indian males with the *li* gene, in the company of which it arrived here, when young, from the West Indies.

VARIABILIS (VA) IN Y.

Two of the original *Lebistes* males brought from the West Indies were of different appearance from those above mentioned, but very much alike one to the other. They were also subsequently found to possess the same gene for colour pattern, viz. "*variabilis*," denoted by *va*.

My reason for naming this gene *variabilis* is, that its phenotypical variation is greater than in any of the other types. Both the number of red side patches and the number and position of black specks, as well as the pattern on the caudal fin, are subject to considerable variation. The most characteristic features are noted in the following description.

variabilis (*va*) in Y (see Plate III, figs. 54 and 55).

  1. Generally 1 to 3 red side patches, the hindmost is the largest, and most of it lies below the lateral line. The foremost is often obliquely above and behind the pectoral fin.

  2. A black speck at the root of the tail, and another on the side of the body, with occasionally a smaller one between them.

  3. A milky, somewhat labyrinthine pattern in the upper part of the caudal fin, which is here inclined to extend out into a point. The extent of the marking varies from a rather narrow border at the upper edge to a pattern covering the whole upper half of the fin, and can in extreme cases also appear on the tail and body.

One of the two *Lebistes* males with the *variabilis* gene was No. 470, and the analysis of this appears in Exp. XII. It was paired, first with an X_0X_0 female, ♀ 553, and again with ♀ 350, which was heterozygous in the sex-linked gene-complex *sulphureus*, which has already been dealt with.

Taken by itself, the little Exp. XII cannot be said to prove one-sided masculine inheritance of the *variabilis* gene, though it does suggest this. On comparing the results with those of Exp. XIII, in which the very similar ♂ 508 is analysed, the mode of inheritance is placed beyond doubt.

As the sons (47 spec.) of the four F_1 males are of the same appearance as the grandfather, ♂ 508, it is evident that the *variabilis* gene is situated in the Y chromosome. Furthermore, the crosses of ♂♂ 891 (see Plate II,

Experiment XII.

♂ **470** shows Y-linked inheritance of the *variabilis* (*va*) gene.

Genera-tion	Parents	Female offspring	Male offspring
F_1	♀ 350 [$X_o X_{su}$] [from other experiment] ⎫ × ♂ 470 ♀ 553 [$X_o X_o$] ⎭ [$X_o Y_{va}$]	theor.: 1 $X_o X_o$: 1 $X_o X_{su}$ theor.: all $X_o X_o$	5 $X_o Y_{va}$ + 2 $X_{su} Y_{va}$ [*applied*: ♂ 607] 4 $X_o Y_{va}$
back-cross	♀ 606 × ♂ 607 [$X_o X_o × X_o Y_{va}$]	theor.: all $X_o X_o$	1 $X_o Y_{va}$

Experiment XIII.

♂ **508** shows Y-linked inheritance of the *variabilis* (*va*) gene.

Genera-tion	Parents	Female offspring	Male offspring
F_1	♀ 507 [$X_o X_o$] ⎫ × ♂ 508 [$X_o Y_{va}$] ♀ 555 [$X_o X_o$] ⎭	theor.: all $X_o X_o$	1 $X_o Y_{va}$ 5 $X_o Y_{va}$ [*applied*: ♂♂ 732, 734, 784 and 786]
back-crosses	♀♀ $X_o X_o$ × ♂♂ $X_o Y_{va}$	theor.: all $X_o X_o$	all $X_o Y_{va}$
back-crosses	♀ 731 × ♂ 732 ♀ 752 × ♂ 734 ♀ 783 × ♂ 784 ♀ 785 × ♂ 786		19 24 [*applied*: ♂♂ 891 and 893] 1 3
later genera-tions	♀ 890 × ♂ 891 ♀ 892 × ♂ 893		6 10
	Total		63 (all $X_o Y_{va}$)

fig. 26) and 893, representing the next generation, show that here also all of the 16 sons have the *variabilis* gene.

In addition to the above-mentioned analysed specimens of *Lebistes*, there were also others, *e.g.* a male brought from the West Indies having a somewhat peculiar appearance, and which, after pairing with an $X_o X_o$ female, produced 10 sons all resembling their father. Here again there was doubtless also a Y gene, but as the analysis was not carried further, there is the theoretical possibility that the specimen was homozygous in regard to an autosomal factor, and the gene effect is therefore not further discussed.

3. THE GENE COMPLEXES *SULPHUREUS* AND *RUBER*. CROSSING-OVER IN BOTH SEXES. LOCATION OF THE GENES.

As noted in the introduction, I have in two previous papers (1922 *b*, 1923) demonstrated crossing-over between the X and Y chromosomes in the male *Lebistes*. In the case of the *elongatus* gene, the position was perfectly clear, in that this gene (*loc. cit.* 1923) fairly frequently, perhaps in something like 4 per cent. of the cases, crosses over from the X to the Y chromosome or *vice versa*.

The crossing-over of the *sulphureus* gene was not quite so clear, only one case of crossing-over being found (*loc. cit.* 1922 *b*). The pairing of an X_oX_o female, ♀ 140, with an $X_{su}Y_{ru}$ male, ♂ 114, of the "Magdeburg race" (see Plate II, fig. 30) produced 45 sons, of which 44 were X_oY_{ru} (see Plate II, fig. 32), as was theoretically to be expected, while one (see Plate II, fig. 33) differed from the rest, exhibiting some *sulphureus* colours and some *ruber*, without having either of these dispositions fully developed. This particular male, which was born in October, 1917, died before it had been paired, and it was thus impossible to carry the matter further. Some subsequent experiments have, however, now shown that the conjecture as to crossing-over was correct. In view of the partial crossing-over of the *sulphureus* and *ruber* genes, I stated, in 1922, that they could not be single genes, but must be of a complex character, and I then gave the following schematic illustration of the X and Y chromosomes in question, and the crossing-over.

Yellow dorsal fin	Uncoloured dorsal fin
Yellow lower side of the tail ...	Red upper side of the caudal fin
	Not yellow caudal fin
Yellow caudal fin	Red spot on the side of the body
Female determining recessive factor	Male-determining dominant factor
Red lower edge of the caudal fin ...	Black side dot on the tail

X   Y
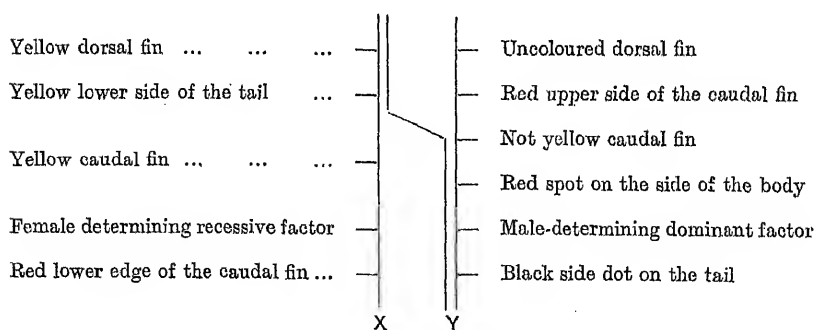
The zigzag line drawn between the X and Y chromosomes indicates the cross-over Y chromosome, which at once shows what must have been the appearance of the divergent *Lebistes* male in question. The animal itself is shown, as mentioned, on Plate II, fig. 33, from the coloured drawing made in 1917.

In March, 1917, another male was born, ♂ 514 (see Plate II, fig. 31), as the product of an entirely similar crossing-over, which here, however, took place in a female individual, namely, the mother of ♂ 514. The pedigree of this animal appears in the accompanying diagram.

March, 1918   ♀ 244 ($X_{su}X_{su}$)   ×   ♂ 189 (X_oY_{ma})
                ("Magdeburg race")   ("Spot race")

April, 1920   ♀ 333 (X_oX_o)   ×   ♂ 334 ($X_{su}Y_{ma}$)
                ("Spot race")

January, 1922   ♀ 413 (X_oX_{su})   ×.   ♂ 383 ($X_{su}Y_{ru}$)
                                                      ("Magdeburg race")

                        ♂ 514 ($X_{o/su}Y_{ru}$)
                        (cross-over-individual)
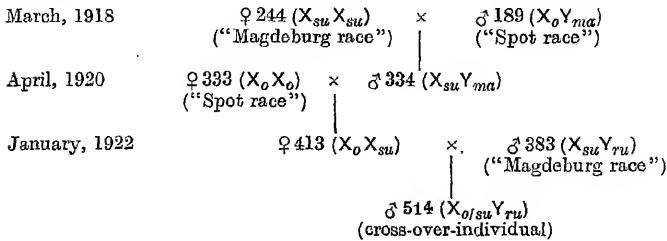
The appearance of ♂ 514 (Plate II, fig. 31) shows that it lacks two of the *sulphureus* characters, viz. the red in the lower part of the caudal fin, and the yellow in the caudal fin; it has, however, the yellow in the dorsal, and in the lower, hindmost part of the tail. All the *ruber* characters are present, viz. the red in the upper part of the caudal fin, the black speck at root of tail and the elongated red spot on the side of the body.

The explanation of this animal's occurrence is clear beyond doubt. In the mother, ♀ 413 X_oX_{su}, a crossing-over has taken place between the X chromosomes, whereby the *sulphureus* complex has partly crossed over to the X_o chromosome. If, in the foregoing diagram of the crossing-over between the X_{su} and Y_{ru} chromosomes, the latter be replaced by an X_o chromosome which, as we know, contains no colour genes, we then obtain, by crossing-over at this very place, the X chromosome which entered into the ovum of ♀ 413 (see accompanying diagram).

Yellow dorsal fin ...   .   —   —0 (Uncoloured)

Yellow lower side of the tail   ...   —   —0 (Uncoloured)

Yellow caudal fin ...   .   —   —0 (Uncoloured)

Female-determining recessive factor   —   —Female-determining recessive factor

Red lower edge of the caudal fin ...   —   —0 (Uncoloured)

X_{su} X_o
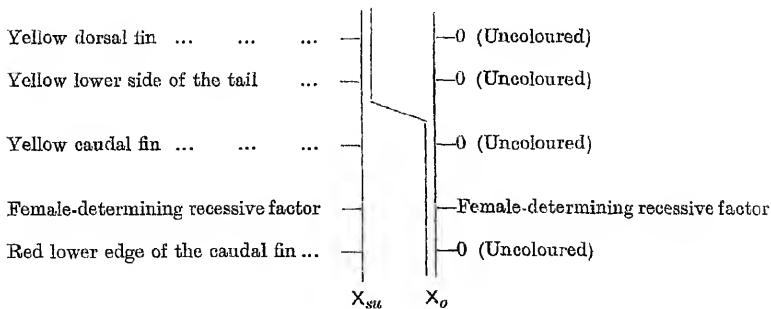
Precisely the same *sulphureus* characters which were lacking in the cross-over specimen from 1917 are lacking in ♂ 514.

Experiment XIV.

Analysis of c/o ♂ 514 $X_{o/su}Y_{ru}$.

Generation	Parents	Female offspring	Male offspring	
F_1	♀ 513 × ♂ 514 [X_oX_o × $X_{o/su}Y_{ru}$]	theor.: all $X_oX_{o/su}$ [applied: ♀♀ 693 and 695]	5 X_oY_{ru}	
back-crosses	♀♀ $X_oX_{o/su}$ × ♂♂ X_oY_{ma}:	theor.: 1 X_oX_o : 1 $X_oX_{o/su}$	X_oY_{ma}	$X_{o/su}Y_{ma}$
	♀ 693 × ♂ 694	[applied: ♀♀ 910, 912 and 914]	18 [applied: ♂♂ 913 and 915]	12
	♀ 695 × { ♂ 696 ; ♂ 708 } [♂ 708 from other experiment]		5 [applied: ♂ 911] 1	8 1
	Total Calculated		24 [22·5]	21 [22·5]
later generations	♀ 910 [$X_oX_{o/su}$] × ♂ 911 [$X_{o/su}Y_{ma}$]	theor.: 1 $X_oX_{o/su}$: 1 $X_{o/su}X_{o/su}$	7X_oY_{ma} + 10 $X_{o/su}Y_{ma}$	
	♀♀ X_oX_o × ♂♂ $X_{o/su}Y_{ma}$:	theor.: 1 X_oX_o : 1 $X_oX_{o/su}$	all X_oY_{ma}	
	♀ 912 × ♂ 913 ♀ 914 × ♂ 915		10 25	
	Total		35 (all X_oY_{ma})	

The control analysis of the cross-over male No. 514 will be seen in Exp. XIV.

The inheritance of the X chromosome arising from the cross-over is, as we see, quite normally sex-linked. The two heterozygous females 693 and 695 have together produced 24 X_oY_{ma} sons and 21 $X_{o/su}Y_{ma}$; theoretically, 1 : 1. The two types are shown in Plate I, fig. 18, and Plate II, fig. 35, respectively.

In the next generation 3 females were paired, viz. ♀♀ 910, 912 and 914, which could theoretically be either X_oX_o or $X_oX_{o/su}$. The first of them was evidently of the formula $X_oX_{o/su}$ as it yielded 10 sons of the $X_{o/su}$ type, and 7 with X_oY_{ma} whereas the two last were X_oX_o females, and produced only X_oY_{ma} sons, 35 in all.

This observation of the fact that crossing-over has taken place in this instance at precisely the same place as in 1917, suggests that the two complex genes, *sulphureus* and *ruber*, are probably not so highly

complex as I was previously inclined to believe, but consist only of two genes each. In any case, there is nothing to prove that they are further divisible into smaller units. Consequently *sulphureus (su)* and *ruber (ru)* must be done away with as terms for units of inheritance, and each replaced by two others. For the future, therefore, the two gene components of the *sulphureus* complex will be designated as *vitellinus (vi)* and *coccineus (co)*, those of the *ruber* complex by *minutus (mi)* and *sanguineus (sa)*. The individual effects of these four genes then appear as follows:

vitellinus (vi) in X and Y (see Plate III, fig. 65).

1. Sulphur yellow colour in the dorsal fin, and a dark speck that only at times is visible in the said fin.
2. Sulphur yellow colour low down in the tail.
3. A small red spot on the side of the tail.
4. Dark side-speck in the tail at the base of the caudal fin.

coccineus (co) in X (see Plate III, fig. 64).

1. Red colour in the lower edge of the caudal fin.
2. Sulphur yellow colour in the caudal fin.

These two genes constitute together the gene complex previously denoted by the term *sulphureus*, which is thus equivalent to *co, vi* (see Plate III, fig. 63).

The two last characters, No. 3 and 4, of the description of the effects of the *vitellinus* gene were not mentioned in my earlier description (1922 *b*) of the gene complex *sulphureus*. As pointed out on p.   , No. 3 was first discovered from observation of hermaphroditic females, No. 4 has appeared to be so regularly present that I consider it most correct to refer to it. The males of the "Magdeburg race" thus, as we see, have the disposition for black speck in the tail both in their X and their Y chromosome. It might be questioned whether this is due to crossing-over between X and Y; but there is no evidence for it.

minutus (mi) in Y and X (see Plate III, fig. 61).

Red colour proximally in the upper edge of the caudal fin.

sanguineus (sa) in Y (see Plate III, fig. 62).

1. Large oblong red side-patch, lying for the most part below and behind the dorsal fin.
2. Dark side-speck in the tail at the base of the caudal fin.

These two genes constitute together the gene complex previously termed *ruber*, which is thus identical with *sa, mi* (see Plate III, fig. 60).

The two instances mentioned of crossing-over for the *sulphureus* and *ruber* complexes can therefore be simplified, as shown in the diagram below:
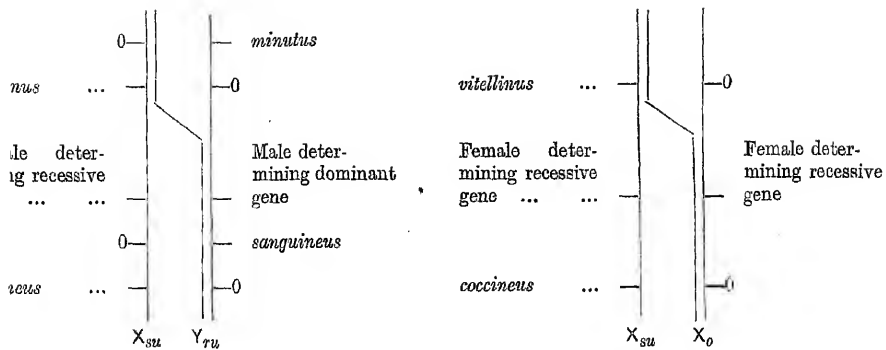
It is possible, however, that the sex-determining gene may be situated not above but below the position noted for *coccineus*. The experiments up to now merely show that this gene is strongly associated with *coccineus* and *sanguineus*, but can be separated from *vitellinus* and *minutus*. I have purposely refrained from placing the genes for colouring in the X and Y chromosomes opposite one another, since nothing is known as to whether they are true allelomorphs; we can only say that they lie at about the same height. All other colour genes hitherto found in *Lebistes* have 0 as allelomorph, and the same is therefore probably also the case here.

4. LINKAGE PHENOMENA IN *LEBISTES*.

The linkage phenomena in *Lebistes* are, as will be evident from the foregoing and from my previous papers, very peculiar. The nearest approach to them is probably found in *Parattetix* and *Apotettix* (Nabours, 1917, 1925). The 18 genes hitherto analysed in *Lebistes* are:

1. *maculatus*	7. *armatus*	13. *luteus*
2. *iridescens*	8. *pauper*	14. *elongatus*
3. *oculatus*	9. *variabilis*	15. *minutus*
4. *ferrugineus*	10. *coccineus*	16. *vitellinus*
5. *sanguineus*	11. *lineatus*	17. *cinnamomeus*
6. *aureus*	12. *tigrinus*	18. *zebrinus*

Of these Nos. 1–9 have always been located in the Y chromosome, and are thus one-sided masculine (Y-linked) in their inheritance; Nos. 10, 11 and 12[1] have always been associated with the X chromosome, and thus show sex-linked inheritance. No. 13 has been found capable of disappearing from the X chromosome by crossing-over (Exp. I), No. 14 can cross over from X to Y and *vice versa* (*loc. cit.* 1923 *a*), No. 15 can disappear from the Y chromosome by crossing-over (the male from

[1] Probably also No. 12 *tigrinus* is able to cross over to Y (see note at Exp. III).

1917 above referred to), No. 16 can cross over from X to Y in the *Lebistes* male (the male from 1917 above referred to) and from X to X in the *Lebistes* female (as mentioned in section 3), No. 17 can occur together with the *pauper* gene in the Y chromosome, but may also be lacking in certain males which have the *pauper* gene, so that it must presumably be capable of crossing-over to X (as previously mentioned in the present work). Finally, No. 18 is found in a pair of autosomes (as shown in Exps. V to VII).

This may be summed up as follows: 9 genes have hitherto invariably been found coupled with the dominant male-determining gene in the Y chromosome, 3 continually with the recessive male determining gene in X (which gene, in accordance with the presence-absence theory, is possibly = O); 5 have shown crossing-over in various ways from X to Y, from Y to X or from X to X, and 1 is associated with a pair of autosomes.

The crossing-over, which seems to be most frequent in the case of the *elongatus* gene, occurring here in about 4 per cent. of cases, is, on the whole, less common in *Lebistes* than in *Drosophila*; and in the case of several genes, as for instance especially *maculatus* and *iridescens* in the Y chromosome, thousands of specimens have been investigated without a single one being found to lack the respective genes. These must therefore, as previously stated (*loc. cit.* 1923 *a*), either be situated so close to the male-determinant gene of the Y chromosome, that crossing-over is practically out of the question, or they must be identical with that gene, which is to say that there must, in *Lebistes*, be multiple allelomorphism in regard to the male determinant factor. It must be left for the future to decide which of these two explanations is correct. It should be added that, owing to the rarity of crossing-over, a very great amount of material will naturally be required before it is possible to assert definitely whether crossing-over can take place or not. It would not therefore be surprising if one or more of the genes I have named were found, on further investigation, to be subdivisible in the same way as the *sulphureus* and *ruber* complexes.

Visitors interested in these *Lebistes* investigations have frequently asked how the allelomorphs of the factors or genes referred to take effect. To this we can only answer that the total of recessive allelomorphs is = O, as further shown in a section in 1923 *a*. Either the particular specimen has one or more of the 18 colour genes, and the effect of these is then constantly seen in all adult males, as if every gene effect were imprinted on the animal—or they lack these genes, and then lack the corresponding colouring. Only when the animals are

in a poor state of health are a few of the characters difficult to discern, as already noted above. In this connection it should be borne in mind that no "empty" Y chromosome has yet been discovered, whereas it frequently happens that an X chromosome contains no colour factors; a point in which *Lebistes* differs markedly from *Drosophila*.

It seems hopeless to enter upon any discussion of the question as to why 17 out of the 18 known colour genes in *Lebistes* are situated in the sex chromosomes, either X or Y, while only 1 of the 22 autosomes has hitherto been found to contain a gene for colouring, and this moreover, of a slight effect, giving the animal a dark striped or barred pattern (*zebrinus*) but no bright colouring. The problem lies beyond the scope of experimental genetics at its present stage, in a region where natural philosophy is as yet able to disport at will.

5. SOME SELECTION EXPERIMENTS WITH THE *LEBISTES* MATERIAL.

As in biological material generally, we can, in *Lebistes*, observe a certain variation, even in specimens of the same genetic constitution, as regards the above-named 18 genes. This variation differs in extent among the different races, but is relatively very slight in most. The greatest variation is undoubtedly found in the effect of the *variabilis* gene, but also in other types which have black specks on body and tail or fins, or red spots and specks in the side, we may find these spots and specks somewhat differently placed, or of slightly different extent, as might have been gathered from the colour plates previously published (*loc. cit.* 1922 *b*).

In regard to this variation, I have, especially for the *maculatus* gene, made some selection experiments with a view to ascertaining whether selection in a given direction produced any effect. On crossing a *maculatus* male, of the formula X_oY_{ma} with females lacking colour genes (X_oX_o), we should expect the result to be as devoid of complications as could well be possible, in that all male offspring will always be of the formula X_oY_{ma}.

The black spot on the dorsal fin (Plate I, fig. 18), which has given its name to the *maculatus* race, varies somewhat both in size and intensity. As a rule it is black and of considerable size, but can be more greyish, or less sharp in outline, and smaller in extent, or, as it were, composed of two or three smaller spots. So also it may in some cases be set obliquely on the dorsal fin, as in Plate I, fig. 2. Only rarely

are any males found with the spot faint or lacking after they are fully grown.

A *maculatus* male, No. 162, was selected for crossing, as it lacked the spot entirely. It was found, however, that it later developed a small double spot. It was paired with two females, one of the *maculatus* race, ♀ 163, which bore 53 sons, of which several had a rather small dorsal spot, and the other an X_0X_0 female of different origin, which bore 21 sons, of which only 8 had the spot strongly developed, the remainder having only a small or fainter spot, often with a tendency to division. From these last, the one with the faintest spot, ♂ 217, was selected and paired with various females of different origin. The first, ♀ 216, of the *iridescens* race, bore 5 sons with rather small spots, the second, ♀ 218, of the *maculatus* race, 5 sons with nearly normal spots; the third, ♀ 219, a sister to ♂ 217, bore two sons almost without dorsal spot; the fourth, ♀ 287, of the *iridescens* race, bore 48 sons with nearly normal but on the whole rather small spots, the fifth, and last, ♀ 145, of the *iridescens* race bore several sons, of which some lacked the spot entirely (see Plate I, fig. 19). A son of ♀ 145, which lacked the spot, was selected from these, and this, ♂ 328, paired with ♀ 327 of the *maculatus* race, produced 9 sons, all without spot even at the age of two years. Paired with another female, viz. ♀ 344 of the *iridescens* race, on the other hand, it produced 6 sons, of which 3 lacked the spot, while 3 had a small spot. It only remains to add that out of 65 F_2 males born of ♀ 145 × ♂ 217, there were 27 without spot, about 25 with small or faint spot, and about 13 with normal spot. The categories were of course not sharply defined.

From this it will be seen that some selective effect has undoubtedly been found to exist, though this does not necessarily mean that the *maculatus* gene itself is changed. It will be noticed that one and the same male can at times give somewhat varying spot intensity in the offspring of two different females, and there is therefore reason to believe that we have here a selective action of "minor factors" affecting the development of the spot; genes which we have not yet been able to analyse further. The occurrence of the black spot on the breast and the black anal speck in *maculatus* specimens can also be affected to some extent by selection, in a somewhat similar manner, though to a lessser degree than noted in the case of the dorsal fin spot. It has been found, however, that the *maculatus* race, which has now been under culture since July, 1916, without selection in any direction, still retains its characteristic appearance.

6. DESCRIPTION OF THE *OCULATUS* AND *FERRUGINEUS* GENES, AND OF VARIOUS COMBINATIONS OF GENES.

As there is no brief description extant of the *oculatus* gene, the Y-linked inheritance of which appears from Schmidt's work (*loc. cit.* 1920), the effect of this is here described in the same way as with the other genes:

> *oculatus* (*oc*) in Y (see Plate III, fig. 50).
>
> 1. Two to four, generally three red, somewhat horizontally elongated side patches, the middle one set low down near the anus, and the foremost high up behind the pectoral fins.
>
> 2. A large black eye behind the pectoral fins, below the foremost red patch (hence the name *oculatus*).
>
> 3. Two to three red flame-like patches in the caudal fin, one above the other, radiating from the base of the caudal fin. The upper margin of the caudal fin has a short sting-like prolongation, often black-edged, in which the uppermost red flame is continued.

A specimen of *oculatus* is shown in Plate I, fig. 21.

The *ferrugineus* gene mentioned in a previous work, which exhibited Y-linked inheritance, but which disappeared from my cultures some years back, together with *oculatus*, whereas the remaining 16 genes are preserved, was, through an error, incompletely described and figured in my previous work (*loc. cit.* 1922 *b*), only the black speck in the tail and the marking of the caudal fin—which are, it is true, the most characteristic features—being used for description of the race. The total effect of *ferrugineus* is as follows:

> *ferrugineus* (*fe*) in Y (see Plate III, fig. 51).
>
> 1. One to four, generally two to three small red side patches, situate mainly in front of the dorsal fin and below it, never far back in the tail.
>
> 2. A black speck in the root of the tail and a similar but larger one behind the pectoral fins or lower down towards the anus.
>
> 3. A large, rust-coloured flame extending from the base of the caudal fin far out into the fin itself, and generally edged with rust-coloured specks.

The other genes dealt with in earlier works have been sufficiently described, but are included for purposes of comparison in the plates accompanying the present work; some male *Lebistes* are also shown as exhibiting various combinations of genes.

The *iridescens* type, for instance, is shown in Plate I, fig. 20. The colour pattern here is due solely to the *iridescens* gene in the Y chromosome, the specific effect of which is shown semi-schematically in Plate III, fig. 49. Concerning the action of the *iridescens* gene it has to be noted that in my earlier description I had left out of consideration a faint yellowish coloration of the upper edge of the caudal fin. As it is nearly

always present, I have found it more correct to show this character in the present figures.

The $X_{co,vi}Y_{ma}$ type, previously designated $X_{su}Y_{ma}$, is shown in Plate II, fig. 34.

The $X_{co,vi}Y_{ma,el}$ type, which has one gene more than the last-named, to wit, the *elongatus* gene, is shown in Plate II, fig. 36.

From Plate II, figs. 42, 43 and 44, it will be seen that males having *elongatus* + *maculatus* are alike in appearance whether *elongatus* is situated in X, in Y or in both chromosomes. The formulae of the three individuals are $X_{el}Y_{ma}$, $X_oY_{ma,el}$ and $X_{el}Y_{ma,el}$ respectively. The specific effect of the *elongatus* gene is seen in Plate III, fig. 69, that of the *maculatus* gene in Plate III, fig. 48.

The *zebrinus* gene is seen combined with *ferrugineus* in Plate II, fig. 45, the formula of the specimen in question being $1_{ze}1_{ze}X_oY_{fe}$.

Finally, the recessive allelomorph for all colour genes, O is shown in Plate III, fig. 70, which thus represents the effect of the X_o chromosome—or its lack of effect.

7. TECHNICAL METHODS OF *LEBISTES* CULTURE.

In view of the fact that *Lebistes reticulatus* has, in the course of years, exhibited such remarkable genetic conditions and is presumably at present the most suitable type of fish for genetic research, I give in the following paragraphs some hints on the culture of *Lebistes*, based on over 12 years' experience.

Temperature. *Lebistes* thrives best at a temperature of 22–26° Celsius, but brief fluctuations down to 19° or up to 30° have no harmful effect. At the low temperature the fish become rather torpid or sluggish; at the high temperature nervous and hasty in movement. At 18° they can hardly be brought to propagate; at 15° they can hardly be kept alive for any length of time. In a bad light the temperature should be kept lower than in strong light, unless artificial aeration is employed so as to give a sufficient supply of oxygen. I do not use this myself.

Water. Ordinary tap water can be used, but should first be heated to boiling point, partly to get rid of the chalk and partly to free it from any disease germs, spores of *Saprolegnia* and the like. It may be dangerous to use natural fresh water from lakes or ponds, as this often brings parasites with it. The boiling of the water can be dispensed with if water from a large aquarium be used for filling the smaller ones. A third of the water in a large aquarium (containing 16–20 litres or more) can be used for this purpose, filling up afterwards with ordinary

unboiled tap water. Old aquarium water becomes yellow with humus
and uric products, and is then ideal, as the development of harmful
organisms is hindered by the yellow water. The water should not,
however, be quite brownish yellow. When an aquarium is to be re-
newed, the water is filtered through cotton and used over again.

Aquaria. For the sake of economy in cost and space (I have at the
present time some 350 aquaria in use) I use, for pairing, two-litre glass
preserving jars. These can also be used for batches of 8–12 males or
3–5 females. A layer of rinsed sand, 5 cm. thick, is laid on the bottom;
a small flowerpot 5 cm. high without hole in the bottom (the hole can
easily be closed with cement) is pressed down into the sand. In the
flowerpot is planted an aquatic plant in good nourishing soil; the soil,
however, only two-thirds the depth of the pot, with sand above. A round
glass plate is laid over the mouth of the jar when filled, to prevent the
fish from jumping out.

For collections of young, and for large numbers of specimens, rect-
angular glass tanks of 4–8–12 litres are used, according to the number
of fish. These tanks are made of flat glass plates set with putty in a
frame of painted zinc, the metal bent to a right angle so that each piece
receives the edge of two glass plates. In the larger aquaria several
flowerpots with aquatic plants are placed.

Glass aquaria cast in one piece are not to be recommended for
practical purposes, as they are liable to crack under change of tem-
perature (especially when being cleaned), in addition to which, the
rougher surface of the glass renders accurate observation impossible.

Aquatic plants. Snails. Plants which can be recommended for this
purpose are *Vallisneria, Sagittaria* and *Ludwigia*; also *Riccia fluitans* in
aquaria where many young fish are born, as it provides good hiding
places for the young. Water snails are most useful for preventing the
accumulation of green and blue-green algae on the glass sides; they also
consume surplus food, and thus prevent it from rotting. Unfortunately
the snails themselves do not as a rule thrive very well, as the *Lebistes*
have a way of nipping off their tentacles. Superficial cleansing of the
glass sides can be effected by means of an old razor blade fixed at the
end of a stick; this is excellent for rubbing off algae. Aquaria placed
directly in sunny windows should be shaded from the strong light in
order to prevent too abundant development of green algae.

Food. Frequent feeding is important; to keep the animals in good
condition, they should be fed twice daily at least. Dry food, however,
among which dried *Daphnia* are especially to be recommended, should

only be given in such quantities as the fish will eat up at once; otherwise it will rot. For small young fish the *Daphnia* should be crushed in the fingers. Live *Enchytreae* should be used to the greatest possible extent. These are cultivated in boxes with good mouldy soil, into which are placed some lumps of rather soft oatmeal porridge, either just under the surface of the soil or on top. The worms, which come up to the porridge, can, in good cultures, be taken up by the thousand with hardly any earth adhering. Before the porridge has been quite eaten up, and the *Enchytreae* have distributed themselves once more throughout the box, a further supply of porridge should be laid down. It is as well to have 3–4 boxes in use. I use 5 boxes, of 60 × 20 cm. and 20 cm. deep, for 300–400 aquaria. The boxes should be closed with a wooden lid and kept in a fairly cool place. *Enchytreae* can be caught in the open by placing oatmeal porridge in an old manure heap. They are a splendid form of nourishment, and involve little risk of over-feeding and subsequent putrefaction, as the worms live some days in the water. They should be cut up for the very young fish. The male *Lebistes* eat far less than the females.

Pairing, pregnancy and birth of young. A male may be allowed to live together with several females, but care should be taken in such cases, as the females will sometimes attack the male and kill it. For the same reason it is better to introduce a female to a male than the reverse. When the male has once got used to the aquarium after a few hours, it will not be frightened by the far larger and stronger female. Generally the male will pursue the female unceasingly, day after day. The period of pregnancy is about 30 days, but can in extreme cases be reduced to 26. After copulation the female is fertilised for a long time, up to 8 months observed, as the spermatozoa remain living in the folds of the ovary; the male, however, is allowed to remain with the female, unless required for pairing elsewhere. The female gives birth to a batch of young each month, exhibiting some restlessness before and during the event. Young females produce as a rule but few young at the first birth, the numbers then increasing, 20–30–50; large females can indeed bring forth over 80 young in one batch. After each birth, which takes from one hour to over 24 hours, as a rule only a couple of hours, the young should be removed as soon as possible, as the females are inclined to eat them up.

Growth of young. Young growing fish thrive best in a spacious aquarium, of 4 litres or more. Males and females should be separated as soon as their sex can be determined, *i.e.* 2–3 weeks before maturity is reached. As a rule, the animals are mature at the age of 3–4 months.

Under ideal conditions, especially with ample space, it may be attained at only 1–2 months; under less favourable conditions often not until they are 6 months old. As a rule, I remove the adolescent males with a tiny hand-net, the adolescent females being allowed to remain in the young-fish aquarium until they are so big that there is danger of their devouring new arrivals of tender age. The males are then gradually assembled in one aquarium, the females in another. *Lebistes* can attain an age of over 3 years, probably as a rule $2\frac{1}{2}$. The female retains her functions until death.

Disease. Epidemics are comparatively rare when rules are properly observed. Over-feeding can at times give rise to a strong development of infusoria, which renders the water slightly milky. The reproduction of these infusoria can take place so rapidly that they soon use up all the oxygen in the water. The fish then come to the surface and remain there gasping for breath, and are suffocated in the course of a day or two, unless the water is changed. In fresh water especially quantities of infusoria may often appear. By washing the fish in several changes of water, thoroughly cleansing the aquarium, and pouring in old aquarium water, however, serious damage can generally be avoided. Occasionally, though not often, one may see fish after fish die in the aquarium, attacked by *Saprolegnia* or some other epidemic disease. Swift and radical measures are then required; disinfection of the aquarium with copper sulphate, ejection of all plants, and transference of the fish to a fresh aquarium. Insufficient supply of aquatic plants in an aquarium hinders the development of the fish, unless artificial aeration is employed.

Drawings of Lebistes. In examining the specimens for the study of genetic conditions, it is advisable to have printed outlines of the male *Lebistes*, on which the colours of the individual specimens can be marked with coloured chalks. I have had a block made with line drawings of 18 *Lebistes*, and use sheets printed from this. Individual specimens are transferred to small flat-sided glass tanks and placed there while drawing takes place; more important specimens are drawn in water colours.

SUMMARY.

A total of 18 genes for colour markings have been found in *Lebistes*, of which nine new ones are described in the present work. The two gene complexes previously described, *sulphureus* and *ruber*, are each composed of two genes, which can be separated by crossing-over, viz. *vitellinus* + *coccineus*, and *minutus* + *sanguineus*.

Of the 18 genes whose effects are shown in Plate III, nine (*maculatus*, *iridescens*, *oculatus*, *ferrugineus*, *sanguineus*, *aureus*, *armatus*, *pauper*, *variabilis*) are located in the Y chromosomes, and three (*coccineus*, *lineatus*, *tigrinus*) in the X chromosome. None of these has hitherto shown crossing-over[1]. Five genes (*luteus*, *elongatus*, *minutus*, *vitellinus*, *cinnamomeus*) have shown crossing-over in various ways, from X to Y, from Y to X or from X to X. Crossing-over thus takes place in both sexes. Finally, one gene (*zebrinus*) is located in a pair of autosomes.

Four older females were altered in a masculine direction and partially furnished with a copulatory organ (*gonopodium*). They developed colouring in accordance with their genetic constitution and contained the gene complex *sulphureus* in X. The sex-glands were hermaphroditic, but when paired with males, the individuals proved capable of functioning as females, and exhibited normal inheritance. In other cases also, females with colour genes—*elongatus* and *zebrinus*—have exhibited male secondary sexual characters.

Selection experiments through several generations with *maculatus* males, $X_o Y_{ma}$, which are particularly characterised by a black spot in the dorsal fin, showed that it was possible to produce almost entirely spotless males, but the spot often recurred, or appeared more pronounced, in the next generation. This must presumably be due to the effect of "minor factors" in the other chromosomes.

The sex-determining gene in the Y chromosome is discussed.

Suggestions are given as to methods of cultivation for *Lebistes*.

[1] The note added to Exp. III. shows that probably also *tigrinus* can cross over to the Y chromosome.

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EXPLANATION OF PLATES

PLATE I.

Fig. 1. $\female\, 721\, 1_o 1_o X_{li} X_o$. Fig. 2. $\male\, 722\, 1_o 1_{ze} X_o Y_{ma}$.

Figs. 3–14. 12 F_1 males of the cross $\female\, 721 \times \male\, 722$; inheritance concerning three genes, viz. *ze* in the first autosome, *li* in X and *ma* in Y.

Fig. 15. Old hermaphroditic $X_o X_{su}\, (=co,\, vi)$ female with *gonopodium* and showing the *co, vi*-pattern.

Fig. 16. Old hermaphroditic female, $X_o X_{su}\, (=co,\, vi)$, showing the *co, vi*-pattern.

Fig. 17. Old hermaphroditic female, $X_o X_{el}$, showing yellow colour in the upper part of the caudal fin, viz. a weak effect of the *elongatus* gene.

Figs. 18–23. Males of different formulae:

Fig. 18. $X_o Y_{ma}$ proper (*maculatus* race). Fig. 19. $X_o Y_{ma}$ of $\female\, 145 \times 217\, F_2$ (no spot in the dorsal fin). Fig. 20. $X_o Y_{ir}$ (*iridescens* race). Fig. 21. $X_o Y_{oc}$ (*oculatus* race). Fig. 22. $X_o Y_{au}$, F_1 of $\female\, 524 \times \male\, 462$ (older individual). Fig. 23. $X_o Y_{au}$, F_1 of $\female\, 690 \times \male\, 691$ (younger individual).

PLATE II.

Figs. 24–47. Males of different formulae (continued):

Fig. 24. $\male\, 769\, X_o Y_{ar}$. Fig. 25. $X_o Y_{ar}$, F_1 of $\female\, 644 \times \male\, 640$. Fig. 26. $\male\, 891\, X_o Y_{va}$. Fig. 27. $\male\, 506\, X_o Y_{pa}$. Fig. 28. $\male\, 501\, X_{li} Y_{pa\, ci}$. Fig. 29. $\male\, 907\, X_{li} Y_{ma}$. Fig. 30. $X_{co,\, vi} Y_{sa,\, mi}$ (the former $X_{su} Y_{ru}$), Magdeburg race. Fig. 31. $\male\, 514\, X_{vi} Y_{sa\, mi}$ ($X_{o/su} Y_{ru}$), cross-over male. Fig. 32. $X_o Y_{sa,\, mi}$ ($=X_o Y_{ru}$), F_1 of $\female\, 140 \times \male\, 114$. Fig. 33. $X_o Y_{sa.\, vi}$, cross-over male, F_1 of $\female\, 140 \times \male\, 114$. Fig. 34. $X_{co,\, vi} Y_{ma}$ ($=X_{su} Y_{ma}$), F_1 of $\female\, 351 \times 355$. Fig. 35. $\male\, 915\, X_{vi} Y_{ma}$ ($=X_{o/su} Y_{ma}$). Fig. 36.
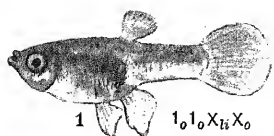
1    $1_o 1_o X_{li} X_o$ 2 $1_o 1_{ze} X_o Y_{ma}$

3    $1_o 1_{ze} X_{li} Y_{ma}$ 4 $1_o 1_o X_o Y_{ma}$ 5 $1_o 1_{ze} X_o Y_{ma}$

6    $1_o 1_o X_o Y_{ma}$ 7 $1_o 1_{ze} X_{li} Y_{ma}$ 8 $1_o 1_o X_{li} Y_{ma}$

9    $1_o 1_{ze} X_o Y_{ma}$ 10 $1_o 1_{ze} X_{li} Y_{ma}$ 11 $1_o 1_o X_o Y_{ma}$

12    $1_o 1_o X_{li} Y_{ma}$ 13 $1_o 1_o X_o Y_{ma}$ 14 $1_o 1_{ze} X_o Y_{ma}$

15    $X_o X_{co, vi}$ 16 $X_o X_{co, vi}$ 17 $X_o X_{el}$

18    $X_o Y_{ma}$ 19 $X_o Y_{ma}$ 20 $X_o Y_{ir}$

21    $X_o Y_{oc}$ 22 $X_o Y_{au}$ 23 $X_o Y_{au}$
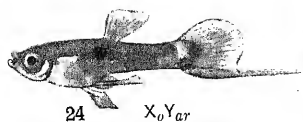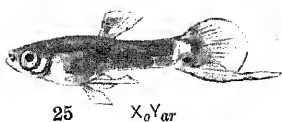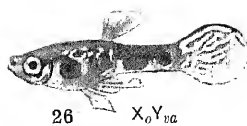
24    $X_o Y_{ar}$ 25 $X_o Y_{ar}$ 26 $X_o Y_{va}$

27    $X_o Y_{pa}$ 28. $X_{li} Y_{pa,ei}$ 29 $X_{li} Y_{ma}$

30    $X_{co,vi} Y_{sa,mi}$ 31 $X_{vi} Y_{sa,mi}$ 32 $X_o Y_{sa,mi}$
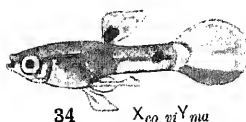
33    $X_o Y_{sa,vi}$ 34 $X_{co,vi} Y_{ma}$ 35 $X_{vi} Y_{ma}$
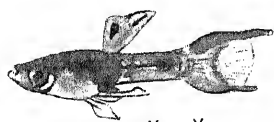
36    $X_{co,vi} Y_{ma,el}$ 37 $X_{ti} Y_{au}$ 38 $X_{ti} Y_{ma}$
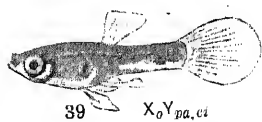
39    $X_o Y_{pa,ei}$ 40 $X_{li,lu} Y_{au}$ 41 $X_{ti,lu} Y_{ma}$

42    $X_{el} Y_{ma}$ 43 $X_o Y_{ma,el}$ 44 $X_{el} Y_{ma,el}$
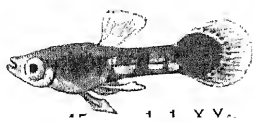
45   46 $1_{ze}1_{ze} X_o Y_{ma}$ 47 $1_o 1_{ze} X_{li} Y_{ma}$

48   *ma* (Y)

49 — *ir* (Y)
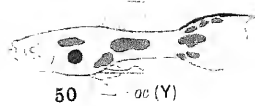
50 — *oc* (Y)
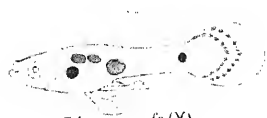
51   *fe* (Y)

52   *au* (Y)

53   *ar* (Y)
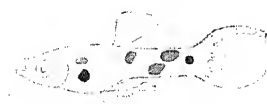
54   *ra* (Y)

55   *va* (Y)

56   *li* (X)

57   *pa, ci*

58   *pa* (Y)

59   *ci* (Y, X)

60   *ru (= sa, mi)*

61 — *mi* (Y. X)

62 — *sa* (Y)

63   *su (= co, vi)*
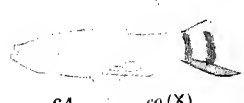
64   *co* (X)
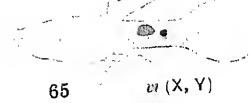
65   *vi* (X, Y)

66   *ti, lu*
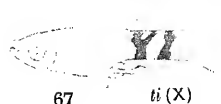
67   *ti* (X)
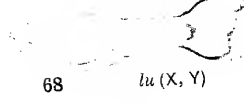
68   *lu* (X, Y)

el (X, Y)
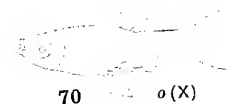
70 — *o* (X)
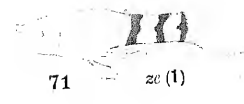
71   *ze* (1)

$X_{co, vi}Y_{ma, el}$ ($=X_{su}Y_{ma, el}$), F_1 of ♀ 652 × ♂ 653.   Fig. 37. $X_{ti}Y_{au}$, son of ♀ 690 × ♂ 691.   Fig. 38. ♂ 875 $X_{ti}Y_{ma}$.   Fig. 39. ♂ 855 $X_o Y_{pa\ ci}$.   Fig. 40. ♂ 440 $X_{ti\ lu}Y_{au}$.   Fig. 41. ♂ 821 $X_{ti,\ lu}Y_{ma}$.   Figs. 42–44.   Three males with the *elongatus* gene connected with the X chromosome, with the Y chromosome and with both the X and the Y chromosome respectively.   Fig. 45. $1_{ze}1_{ze}X_o Y_{fe}$ male.   Fig. 46. ♂ 369 $1_{ze}1_{ze}X_o Y_{ma}$.   Fig. 47. $1_o 1_{ze}X_{ti}Y_{ma}$, F_1 of ♀ 721 × ♂ 722.

PLATE III.

Figs. 48–71.   The effect of the individual genes and of some gene combinations.

ar = armatus.	*mi = minutus.*
au = aureus.	*oc = oculatus.*
ci = cinnamomeus.	*pa = pauper.*
co = coccineus.	*sa = sanguineus.*
el = elongatus.	*ti = tigrinus.*
fe = ferrugineus.	*va = variabilis.*
ir = iridescens.	*vi = vitellinus.*
li = lineatus.	*ze = zebrinus.*
lu = luteus.	*o* = the recessive allelomorph
ma = maculatus.	of all these genes.

STUDIES ON HETEROGONIC GROWTH. (IV) THE BIMODAL CEPHALIC HORN OF *XYLOTRUPES GIDEON*.

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(With Two Text-figures.)

In 1892 Bateson and Brindley pointed out that the males of the widely distributed beetle *Xylotrupes gideon* resembled the males of the Earwig (*Forficula*) in having an appendage (in this case the cephalic horn) whose length showed a bimodal frequency-curve, while the body-length (as measured by length of elytron) was unimodal in its variation.

These authors gave the frequencies for horn-length and body-length separately, and did not publish their measurements in such a way as to make it possible to correlate the two variables. Luckily, however, Mr Brindley kept all the original data, and on my request very generously turned them over to me. I would like here to acknowledge his kindness in doing so, and in answering various questions concerning the material.

The present paper is based chiefly on this material (316 available specimens) and also upon 37 specimens in the Hope Collection, which Professor E. B. Poulton, F.R.S., kindly allowed me to measure myself. The measurements have been made to 0·5 mm. The 353 specimens for which both elytron- and horn-measurements were available have been arranged in a correlation table (Table I).

This table has been condensed by combining the classes both ways by pairs to give Table II. From this, Fig. 1 has been prepared, in which the horn-length frequencies have been plotted for each class of Table II. The solid lines indicate Bateson's and Brindley's material, the dotted lines that from the Hope Department.

From Tables I and II and Fig. 1, it will be seen at a glance that the statement that *Xylotrupes* shows bimodal variation of its cephalic horn needs some qualification. The bimodality only exists for individuals of a certain body-size. The smallest individuals are all of the "low" type as regards horn, the largest all of the "high" type.

TABLE I.

Cephalic Horn-length, man.

Blytron length, mm.	Totals
20·5	1
21·0	1
21·5	1
22·0	6
22·5	11
23·0	12
23·5	10
24·0	19
24·5	18
25·0	18
25·5	14
26·0	17
26·5	27
27·0	22
27·5	33
28·0	24
28·5	23
29·0	21
29·5	14
30·0	12
30·5	11
31·0	8
31·5	6
32·0	1
32·5	1
33·0	1
Tls.	314

Cephalic Horn-length column totals: 24·0 → 1; 23·5 → 3; 23·0 → 3; 22·5 → 3; 22·0 → 27; 21·5 → 3; 21·0 → 8; 20·5 → 14; 20·0 → 10; 19·5 → 14; 19·0 → 7; 18·5 → 11; 18·0 → 10; 17·5 → 8; 17·0 → 11; 16·5 → 3; 16·0 → 10; 15·5 → 6; 15·0 → 5; 14·5 → 2; 14·0 → 5; 13·5 → 4; 13·0 → 5; 12·5 → 8; 12·0 → 7; 11·5 → 9; 11·0 → 13; 10·5 → 1; 10·0 → 12; 9·5 → 8; 9·0 → 12; 8·5 → 18; 8·0 → 15; 7·5 → 14; 7·0 → 7; 6·5 → 15; 6·0 → 5; 5·5 → 1; (last) → 1.

TABLE II.

Class Centres. Cephalic Horn-length, mm.

Pygidium length, mm.	1·75	2·75	3·75	4·75	5·75	6·75	7·75	8·75	9·75	10·75	11·75	12·75	13·75	14·75	15·75	16·75	17·75	18·75	19·75	20·75	21·75	22·75	23·73	Totals A	B
15·75	(1)																							—	1
16·75		(1)																						—	1
17·75		(1)																						—	1
18·75			(3)																					3	3
19·75				1(1)	1	1																		3	4
20·75				1(3)	4	3			1	1														8	11
21·75			(1)		1	15	5	2	5	4	1	1												28	24
22·75				(1)		3(1)	17(1)	7	6	3(1)	7	3+1	3	2	4	3		1						29	32
23·75					(1)	2	7	12	8	3(1)	4(1)	3	5	3	8	4(1)	10	4	2					32	33
24·75								9(1)			3(1)	5	1	2	3	3	4	8(1)	3	3				44	46
25·75						(1)									1	1(1)	3	4	8(1)	12	3(1)			55	57
26·75								(2)				(1)				4(1)	4	1	5	5	5	1		47	57
27·75							(1)						(1)				3	1	1	2	5	1		35	35
28·75							(1)				(2)		1			1(1)					10	1		23	27
29·75									(1)			(1)									6	3	2	14	16
30·75																					(1)		2	2	4
31·75									(1)													1		4	1
32·75																								1	1
Totals A	—	—	—	2	6	24	29	30	20	14	16	13	9	7	16	14	18	18	24	22	24	6	4	**316**	
B	1	2	4	7	7	26	32	33	22	16	20	14	10	7	16	16	16	19	25	22	26	6	4		**353**

The material of Table I, re-grouped by 1 mm. classes, and with the Hope Department material added in brackets ( ).

Total A=Bateson's and Brindley's material. B=All specimens.

In Bateson's and Brindley's material all body-classes up to 235 mm. inclusive are entirely "low," all from 29·0 mm. upwards are entirely "high." The 24·0 and 25·0 mm. classes have only one high horn each, the 28·0 and 28·5 mm. class only one low horn apiece.

The Hope Department material has a considerably lower mean horn-size. When this is included with the rest, the bimodal body-classes, instead of ranging only from 24·0 to 28·5 mm., reach up to 31·5 mm. However, above 30 mm., the bimodal classes only boast one low specimen apiece.

In this respect, of the bimodality of the appendage being limited to the centre of its range as regards absolute body-size, *Xylotrupes* resembles *Forficula* (see Huxley (1927)). This (together with the body's unimodality) makes one suspect that the bimodality depends not upon genetic variations but upon the existence of "equilibrium-positions" of horn-length which are most readily adopted during development.

It is further clear from inspection that the two modes are by no means constant, but are in positive correlation with body-size. When the mean horn-length is calculated for each body-class :—(1) for all specimens in the class, (2) for all low-type, and (3) for all high-type specimens, the results set forth in Table III are obtained. It is not always certain to which type the intermediate horns should be assigned. The assignment I have made is indicated in Tables I and II. When the means thus obtained are plotted on a double logarithmic grid, Fig. 2 is obtained. It is clear at once that, fundamentally, the cephalic horn of *Xylotrupes* is heterogonic, and that its relative growth approximates to that expressed by the formula $y = bx^k$ (see Huxley, 1924) where y = horn-length, x = elytron-length, and b and k are constants. This certainly holds good for the body-classes from 16·0 to 25·0 mm. inclusive. Not only this, but the heterogony is marked, k being about 2·75, which is a very high value (the value would probably be considerably less if volumes could be measured in place of lengths).

When, however, we reach the markedly bimodal classes (25·5 to 27·5 mm., body-length inclusive) we find a very different state of affairs from that seen in *Forficula*. In the first place, the bimodality is not nearly so sharply marked as for the earwig-forceps. In Fig. 1 it is at once seen that the curves for body-classes 25·75, 26·75 and 27·75 mm. are irregular, with a tendency to show one or more extra modes between the main "low" and "high" modes. This was not the case in *Forficula*. In addition, if the means for low and high as well as totals for each body-class are plotted, it is seen that the means for totals rise to give a con-
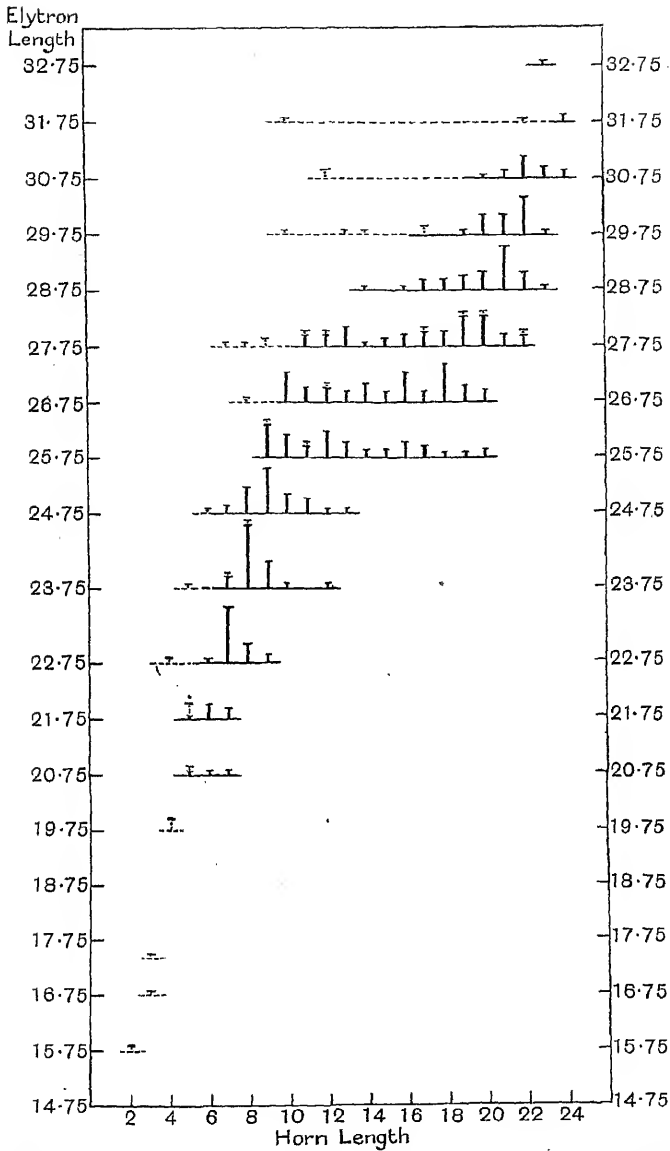
Fig. 1. Frequencies of horn-lengths for each body-length class. Unbroken lines = Bateson's and Brindley's material. Dotted lines = Hope Department material. It will be seen that marked bimodality only exists near the centre of the body-length range.

siderably higher value of k (as indicated by greater steepness of slope) until 28·75 mm. body-length is reached. The means of the "high" horns, further, show a positive heterogony, with k considerably greater than 1, all the way, although the slope of their curve is not constant, but decreases gradually. The means for the low types, on the other hand, bend right over and show negative heterogony with k only about 0·75.

It is, however, as I have said, very hard to be sure of one's assignment of the intermediate horns to high or low types. Further, with increased quantity of material from a single locality, it might well prove that the medium body-classes were not really bimodal at all, but that they were tri- or quadrimodal. In this case, the original "low" types would be represented only by the very low modes, *e.g.* at 9 and 10 mm. in the body-classes at 25·75 and 26·75 mm. (Fig. 1), while the subsidiary modes really did represent further true developmental "equilibrium-positions." In this case, we should obtain three or more lines of mean horn-length in the mid-ranges of body-size, all exhibiting negative heterogony and running approximately parallel with each other, the main effect of increase of body-size being to increase the percentage of individuals in the "higher" equilibrium-positions. If this were so, *Xylotrupes* would come to resemble *Forficula* more nearly, but with the additional complication of several equilibrium-positions, instead of only two. Without large numbers, and especially without experiments on altering the size of the larvae at pupation, it is impossible to decide between this alternative and that of great irregularity of variation at medium body-sizes.

In any case it is worth noting that at the highest body-sizes, the values for total means again come to lie almost exactly on a continuation of the original curve for the low total means (see Fig. 2); this indicates that most probably, as in *Forficula*, the basic developmental mechanism for the cephalic horn of *Xylotrupes* is a simple heterogonic one, providing for a constant differential ratio of the growth-rates of horn and body (as *e.g.* in *Uca*—see Huxley, 1924); but that this is masked by the secondary complication of equilibrium-positions (see Huxley, 1927).

It is difficult to understand why the specimens from the Hope Collection should mostly exhibit relatively smaller horns than those in Bateson's and Brindley's material. It is much to be wished that further work should be done on this remarkable insect. In the first place, large collections should be made in various localities, and, if possible, in some localities for several years in succession. In the second place, experiments should be made to try to control the development of the horn. It may
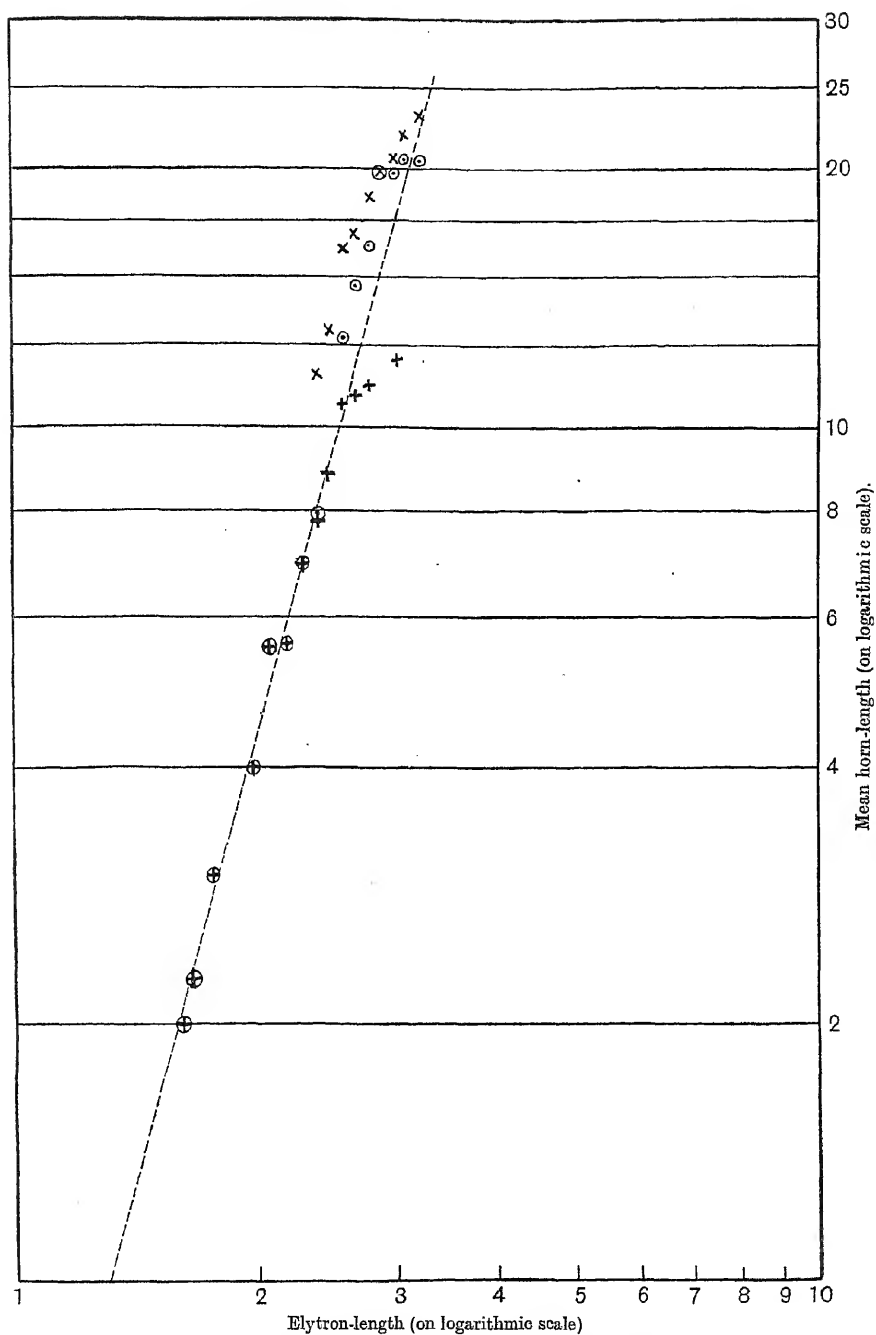
Fig. 2. Relation between elytron-length and horn-length.
⊙ all specimens.   + "low" type.   × "high" type.

be suggested (1) that generally different conditions throughout the larval period would be effective, (2) that even more demonstrative might be the inducing of early metamorphosis at a smaller size than normal by means of partial or total starvation in late larval life.

In any case, I think that this additional analysis of Bateson's and Brindley's data renders it probable, though not certain, that the problem of the bimodality of the horn-length of *Xylotrupes* is primarily developmental and not genetic in nature.

SUMMARY.

(1) A correlation table has been prepared showing the relation between elytron-length and cephalic horn-length in 353 males of the beetle *Xylotrupes gideon*. Of these, 316 specimens are from Bateson's and Brindley's measurements, from material all collected in Sarawak by Baron von Hügel; and 37 are from material in the Hope Collection at Oxford, from various localities, measured by myself.

(2) The bimodality of the horn-length is seen not to exist throughout the whole size-range of the species. The smallest males have unimodal horns of "low" type, the largest have unimodal horns of "high" type. Only the intermediate sizes show a bimodal curve for the horns, the bimodality being marked only for body-length classes 25·5 and 27·5 mm. inclusive.

(3) The bimodality even of these classes is not so clear-cut as for the forceps of male *Forficula*, there being a tendency for three or more modes to exist.

(4) The values of the modes for low and high types increase with increasing body-size; the mean for the extreme high types shows positive heterogony.

(5) When the means for *all specimens* of each body-class are taken, it is found that the relation between them and body-size can be expressed very nearly by the formula $y = bx^k$, which is that for simple heterogony when organ (y) and rest of body (x) exhibit growth-rates whose ratio (k) remains constant, and is found to hold in other Coleoptera, *e.g.* for the mandibles of male Lucanidae. k here is unusually large (about 2·75).

(6) The curves are not so regular for those body-classes whose horns show marked bimodality. It is suggested that possibly over this part of the range there may be not two equilibrium-positions for the appendages (as in *Forficula*), but several, so that the curve of variation here is multimodal, not bimodal.

(7) The general conclusion is drawn that (as is almost certain for the forceps of male *Forficula*) the size of the cephalic horn is probably determined by two developmental mechanisms: (*a*) an underlying mechanism for simple positive heterogony; and (*b*) the existence over part of the range of body-size, of two (or more) equilibrium-positions into which the developing horns tend ·to fall, the effect of increasing body-size being to transfer a larger proportion of individuals from "low" to "high" equilibrium-position.

(8) It is recognised that only further work can fully solve the problem, and the desirability is emphasised of making and measuring large collections of *Xylotrupes* from single localities and, especially, of controlling horn-development by means of experiments on the larvae.

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ON THE EXISTENCE OF EGG-LAYING CYCLES IN THE DOMESTIC FOWL.

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INTRODUCTION.

THE object of this paper is to present a series of preliminary data on the existence of definite egg-laying cycles in the domestic fowl. The results were obtained in the course of investigations, still incomplete, of the genetics of egg-laying capacity. The experiments are unique in one respect, in that they were carried out in a tropical country (Trinidad, British West Indies), where environmental conditions are extraordinarily uniform throughout the year, in respect of both temperature and length of day. It is surprising that previous writers on the inheritance of fecundity in poultry (Pearl, Goodale and others) have failed to take into account the fact that the huge range in temperature and length of day in the North Temperate Zone provide quite peculiar conditions, under which the genetic factors involved can hardly find full expression. In connection with Pearl's hypothesis of a winter cycle in the Barred Plymouth Rock, one may very pertinently enquire how the genes manifest themselves under a uniform temperature.

THE EXPERIMENTAL RESULTS.

The experimental results consist of trap-nest records for varying periods of a mixed lot of fowls comprising Trinidad Natives, and F_1 hybrids between these and Leghorns, Minorcas and Plymouth Rocks. The full records are presented in Tables I–X and the salient points discussed.

NOTES ON THE TRAP-NEST RECORDS.

Trinidad Native, No. 1. It will be seen from this record that in the eight months there are eight cycles of laying, each followed by a broody period. The mean number of eggs per cycle is 11·7, and the mean length of the broody period is 14·3 days. The mean number of days occupied by a clutch and the subsequent broody period is 29·3 days—about a

TABLE I.

Trap-nest Record. Trinidad Native, No. 1.

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
June	—	—	1*	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	—	1	1	—	1	1	1	1	—	1*	9
July	—	1	1*	1	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1*	—	10
August	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	1	—	—	1	1	1	1	1	—	1	1	1*	1*	—	12
September	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	1	—	—	—	1	1	1	1	1	1	1	1*	1	1*	12
October	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	—	1	—	1	1	1	1	1	—	—	1*	1	1*	1	—	11
November	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	1	1	—	1	—	1	1	—	1	—	1	—	—	1	—	12
December	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1*	1	—	1*	—	15
January	—	—	—	—	—	—	—	—	1	—	—	1	—	1	—	—	—	1	—	—	—	—	—	—	—	—	1	1	—	1*	—	11

* Went broody.

TABLE II.

Trap-nest Record. Trinidad Native, No. 2.

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
October	—	—	—	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	—	1	1	1	1	1	1	1	1	1	—	1	1	23
November	—	1	1	1	1	1	—	—	1	1	1	1	1	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	10
December	1	1	1	1	1	1	—	—	1	1*	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	13
January	1	1	—	1	1	1	—	1	1	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8

* Went broody.

TABLE III.

Trap-nest Record. Trinidad Native, No. 3.

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
October	—	1	—	1*	1	1	1	1	1	1	1	1	1	—	—	1	1	—	1	1	1	1	1	—	1	1	1*	—	1	1	—	1
November	1	1	1	1*	1	—	1	1	1	1	1	1	1	—	—	1	1	1	1	1	1	1	1	—	1	1	1	—	1	1	—	21
December	1	—	1	1	1	—	1	1	1	1	1	—	1	—	—	—	1	—	1	1	1	1	1	—	1	1	1*	—	—	1	—	15

* Went broody.

TABLE IV.

Trap-nest Record. Trinidad Native, No. 4.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
October	1	1	1	—	1	1	1	—	—	—	1	1	1	1	1	1	—	1	—	1	1	1	1	1*	1*	—	—	—	—	—	—	18
November	1	—	1	—	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	—	1	1	—	1*	—	—	—	—	—	—	15
December	—	—	—	—	—	—	1	1	1	1	1	1	—	—	—	1	—	—	—	—	—	—	1	1*	—	—	—	—	—	—	—	10

* Went broody.

TABLE V.

Trap-nest Record. Trinidad Native, No. 5.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
October	1	1	1	—	1	1	—	1	1	—	1	1	—	1	1	1	1	1	—	1	1	1	1	1	1	1	1	1*	—	1	—	20
November	1	1	1	1	—	1	—	—	1	—	1	1	—	1	1	1	1	1	—	1	1	1	1	1	1	—	1	1	—	—	—	10
December	—	1	—	1	—	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1*	—	—	9

* Went broody.

TABLE VI.

Trap-nest Record. Black Minorca × Trinidad Native, No. 6.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
April	—	—	—	1	1	1	1	1	1	1	1	—	1	—	1	—	—	—	1	—	1	1	1	—	—	—	1	—	1	1	—	17
May	1	—	1*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	—	—	1	—	—	—	—	—	1	1	—	1	16
June	1	—	1	—	—	1	—	—	—	1	1*	1	—	1	—	—	1	—	—	—	—	1	1	1	—	1	1	1	—	1	1	5
July	1	—	1	1	1	1	1	1	1	1	1	1	1	1	1	—	—	1	1	—	—	1	1	1	—	1	1	1	1	—	—	13
August	1	—	1	1	—	1	—	1	—	1	1*	1	1	1	1*	—	—	—	—	—	—	1	1	1	—	1	1	1	—	—	—	11
September	—	—	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	—	—	—	1	—	—	—	—	—	1	1	1	1	12
October	1	—	1	1	—	1	—	1	1	1	1	1	1	1	1	1	1	1	1	1	—	1	1	1	—	1	1	1	1	—	1	4
November	—	1	1	1	—	1	—	—	1	1	1	1	1	1	1	1*	1	1	1	1	—	1	—	—	—	—	1	—	1	—	—	19
December	—	1	1	1	—	1	—	—	—	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1*	—	—	—	—	—	1	13
January	—	1	—	—	—	—	—	—	1	1	1	1	1	1	1	1	1	1	1	1	1	—	1	1	1	1	—	—	—	—	1	17

* Went broody.

TABLE VII.

Trap-nest Record. White Leghorn × Trinidad Native, No. 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
February				1				1	1		1														1	1	1		1	1		4
March	1		1	1		1		1	1							1	1	1		1	1	1	1	1			1	1		1		12
April	1		1		1	1	1													1*	1	1		1						1		14
May					1								1						1												1	5
June		1		1		1																						1				1
July		1			1					1		1		1					1													10
August							1					1			1	1*		1	1	1			1	1		1			1			3
September		1		1		1	1		1	1	1	1		1	1	1	1	1	1	1	1		1							1		14
October	1		1		1	1	1		1	1	1	1																1	1	1	1	19
November						1	1										1	1	1*	1	1											7
December					1		1	1	1	1	1	1		1	1	1	1	1		1												16
January	1		1		1		1		1																							7

* Went broody.

TABLE VIII.

Trap-nest Record. White Leghorn × Trinidad Native, No. 2.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
February				1		1			1		1	1	1				1	1	1	1	1						1		1			8
March		1		1		1	1	1	1			1	1	1*						1	1	1						1				6
April			1			1						1	1	1	1		1	1		1	1	1	1*	1		1	1			1	1	11
May	1		1										1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	10
June					1	1	1		1	1*	1						1*	1									1	1	1	1	1	11
July		1	1	1	1	1				1*															1		1	1				4
August		1	1		1	1	1	1		1*	1										1	1	1	1		1	1	1	1	1		12
September	1	1	1	1	1	1				1	1*																		1		1	15
October	1	1			1	1	1	1																	1							15
November	1		1		1			1			1*										1		1								1	14
December	1				1																											12
January	1		1		1		1	1													1				1				1			10

* Went broody.

TABLE IX.

Trap-nest Record. Plymouth Rock × Trinidad Native.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
April	—	—	1	1	—	1	1	1	—	1	1	1	1	1	1	—	1	—	1	1	1	—	1	1	1	—	1	—	1	1	—	20
May	1	1	1	1	1	1	—	1	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	9
June	—	—	—	1	1	1	—	—	—	1*	—	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	7
July	1	1	1	1	1	1	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8
August	1	1	—	1	1	1	1	1	1	—	1*	—	1	—	1	—	—	—	1	—	—	1	1	1	1	1	—	1	1	1	—	9
September	1	1	1	—	—	—	1	1	1	—	—	1	—	1	—	1	—	1	1	—	—	—	1	1	1	1	1	1	1	1	—	10
October	1	1	1	1	1	1	1	1	1	1*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10

* Went broody.

TABLE X.

Trap-nest Record. White Leghorn, Pure Bred.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total for month
September	—	—	—	—	—	—	1	—	—	—	1	—	1	—	1	—	1	—	—	1	—	1	1	—	1	—	1	1	—	1	—	5
October	1	1	1	1	—	1	1	1	1	1	—	1	1	1	1	—	1	1	—	1	—	1	1	—	1	1	1	1	1	1	1	20
November	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	—	1	—	1	1	—	1	1	1	—	1	—	—	1	—	—	20
December	1	1	1	—	1	1	1	1	1	—	1	1	1	1	1	1	—	1	1	1	1	1	1	1	1	—	died	—	1	1	—	22
January	1	—	1	1	1	1	1	1	1	—	1	1	1	1	—	1	—	1	1	—	—	1	1	—	—	1	—	—	—	—	—	19

lunar month. It will be noted also that there is consequently consider-
able uniformity in the day of the month at which the hen went broody—
viz. 31 July, 30 August, 29 September, 31 October, 29 November,
27 December, and 30 January.

Trinidad Native, No. 2. This bird began laying in early October
after a long period of moulting. She laid 31 eggs followed by two clutches
of 11 and 12 respectively. No further records were taken, as the bird
was allowed to incubate. The number 31 is suggestive of a triple clutch.
The mean number of eggs per clutch, 11·5, and the mean length of the
broody period, 14 days, are almost exactly comparable with No. 1.

Trinidad Native, No. 3. This was a pullet which laid her first egg on
30 October. She laid 37 eggs, up to 27 December. This is suggestive
again of a triple clutch (3×12), but is of interest again in that the
bird was distinctly broody on 5 December. The number of eggs laid up
to that date was 25, a double clutch of 12×2. The triple clutch is thus
built up of a double clutch and a single clutch.

Trinidad Native, No. 4. This bird is chiefly interesting in the dates
at which broodiness occurred, 24 October, 25 November, and 25 De-
cember, providing further evidence of a monthly cycle of broodiness.
The two broody periods are each 11 days, and the mean number of eggs
per clutch 14·3.

Trinidad Native, No. 5. This bird began laying on 1 October after
moulting. She laid a double (?) clutch of 20, a single clutch of 13, and
a half clutch of 6. It is not improbable that the first clutch of 20 con-
sisted of a single plus a half clutch. The mean length of the two broody
periods was 16·5 days. This bird was probably two years old when
bought, and it is possible that with increasing age native birds tend to
produce half clutches.

Black Minorca × Trinidad Native, No. 6. A batch of 34 eggs is
followed by clutches of 8, 16, and 15. A moulting period of 42 days is
then followed by a batch of 36 eggs, and a further batch of 17. It is
clear that this bird differs from those previously discussed, and the
results can most easily be explained by assuming that the basic clutch
number is 16, and that the eggs recorded comprise two double clutches
(34 and 36), two single clutches (15, 16) and a half clutch (8). The onset
of moulting (16 September) coincided with the commencement of a broody
period.

White Leghorn × Trinidad Native, No. 1. This bird differs from the
preceding ones, in that the non-broody habit of the Leghorn behaved
as a partial dominant. Signs of broodiness were exhibited on many

occasions, but were not accompanied by any pronounced tendency to sit. During the months of May, June, July and August the bird was in poor health, and suffered a good deal from colds. There is a single clutch of 14 eggs ending with a short broody period on 20 March, followed by a half clutch of 6. The rest period between 7 and 16 April was not accompanied by any broody symptoms. Another single clutch of 14 was laid between 17 April, and 14 May, but by this time the bird was obviously affected by respiratory trouble and her behaviour to 28 August must be regarded as abnormal. A single clutch of 13 begins on 29 August, and this is followed by a double (?) clutch of 23, a half clutch of 7, and another double (?) clutch of 23. In the latter clutch slight signs of broodiness were apparent with the 10th egg on 19 December.

White Leghorn × Trinidad Native, No. 2. The types of clutch in serial order are: single clutch (14), double clutch (21), single clutch (11), half clutch (4), double clutch (20), single clutch (15), single clutch (14), single clutch (13), single clutch (13). Leghorn influence was apparent in the less pronounced signs of broodiness.

Plymouth Rock × Trinidad Native. This bird began with a double clutch (28), and followed with a half clutch (6), half clutch (8), single clutch (11), and double clutch (20). She was then allowed to incubate.

White Leghorn, Pure Bred. No cycles are apparently visible.

DISCUSSION AND SUMMARY.

Even with the somewhat meagre series of results presented in this paper the following points are brought out:

1. There is a basal clutch number which is most commonly about 12.

2. The basal clutch number appears in one case to be 16.

3. A bird may lay a series of single clutches, or may lay multiples or sub-multiples of the basal clutch number.

4. When beginning to lay (a) for the first time, (b) after bringing up a flock of chickens, or (c) after moulting, it is most usual to lay some multiple of the basal clutch number. In general, multiples of the basal clutch number occur after a long rest period.

5. The clutch habit is absent in pure-bred poultry of American or European descent. This conclusion has been arrived at by an examination of the trap-nest records at the Trinidad Government Farm, where flocks of White Leghorn, Rhode Island Red, and Barred Plymouth Rock have been trap-nested for several years.

6. In four cases the clutch habit behaves as a dominant, in crosses of Native by Leghorn, Minorca or Plymouth Rock.

7. The time occupied by a single clutch and the subsequent broody period is about a lunar month.

These results are not without significance for breeders of poultry in temperate climates. It may not be too bold a speculation to suggest that the evolution of the domestic fowl in regard to the number of eggs laid per annum has taken place by a series of mutations which have resulted in the partial or complete elimination of the rest periods between clutches or in the production of higher multiples of the single clutch.

The writer is at present engaged on the genetic analysis of the clutch habit in crosses of Leghorn by Native, and interesting results are awaited.

CHROMOSOMES AND SEX IN *VALLISNERIA*.

By C. A. JØRGENSEN.

(*Lyngby, Denmark.*)

(With Ten Text-figures.)

On a joint visit to the Royal Botanical Gardens at Kew in the summer of 1925 Mr W. C. F. Newton of the John Innes Horticultural Institution and the author happened to come across some very big and vigorous Vallisnerias, grown under the name of *V. gigantea* Graebn. In the light of the recent investigations on sex-chromosomes in plants by Kihara and Ono (1923), Sinoto (1924), Blackburn (1924), Winge (1923) and Meurman (1925), we both thought it interesting to look at these *Vallisneria*-plants. It may be mentioned here that the mechanism of sex-determination in *Vallisneria* according to Winge (1923) is unique in the vegetable kingdom. An *XO-(Protenor)* type is found, the male plants being heterogamous and giving pollen grains with an *X*-chromosome (having a definite constriction) and pollen grains without, whereas the females are supposed to be homogamous and the egg cells to contain always an *X*-chromosome. On the basis of his counts in the pollen grains Winge gives the diploid numbers in male and female Vallisnerias as 17 and 18 respectively, or 18 and 20, if the two portions of the *X*-chromosome should be apart in the somatic divisions (1923, p. 11).

The *Vallisneria gigantea* plants were all males. Supposing them to be tetraploid we expected to find 34 chromosomes in their root tips. In the slides however 40 chromosomes were present. This introduced a puzzling element, and in order to follow up the problem definitely we both made fresh fixations in the following summer. The descriptions here are based upon Mr Newton's slides as well as on my own. I am especially indebted to Mr Newton for material of the reduction division in *V. spiralis* as my own material happened to fail on this point. The fixations were mostly in Flemming's solution and the material stained in gentian-violet-iodine (by a modification of Gram's method for staining bacteria), the practical application of which method I also owe to Mr Newton.

I: *Somatic divisions in* V. spiralis *L. and* V. gigantea *Graebn.*

The divisions in the root tips show the chromosomes very well if the material is fixed and stained properly. Many plates have been counted in *V. spiralis* and in all cases where a definite statement was possible, 20 chromosomes were found (Fig. 1).
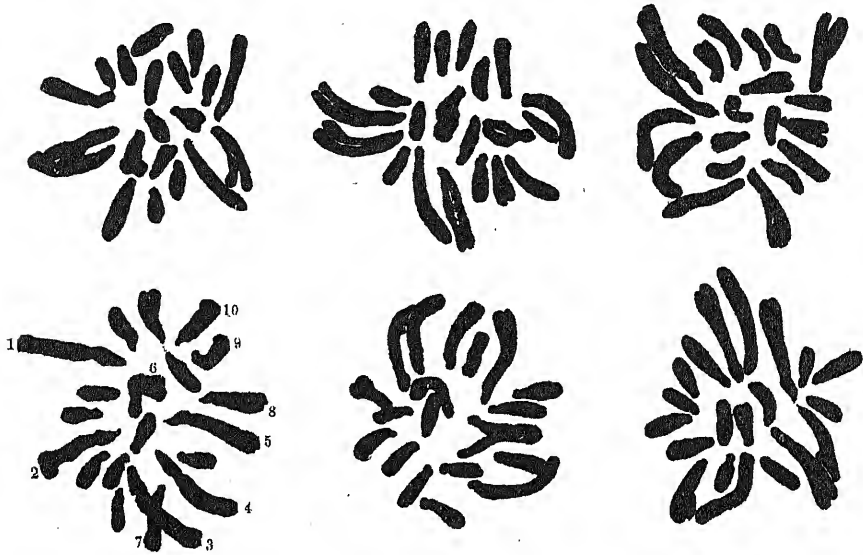

Fig. 1. Metaphase plates from root tips of *V. spiralis* ♂. (×2500.)

As seen from the figures, the chromosomes differ much in size, some being very long, others intermediate, and others again quite small. Most of the chromosomes take up a radial position in the plate. The end of a chromosome pointing naturally towards the centre is called the proximal one, the other the distal one. All the chromosomes have a definite constriction at the proximal end. In the small and medium-sized chromosomes the constriction cuts off an almost spherical body from the rest of the chromosome, whereas in the 6 long chromosomes the constriction is farther from the end, and cuts off a cylindrical piece which is often pointed (see Fig. 1). The subterminal constrictions give the pieces the character of satellites or trabants, but their connection with the main part of the chromosome is always a very close one. Similar constrictions have been previously described in a number of plants (mostly mono-cotyledons) and are also known to occur in insects. Different investigators explain these constrictions in different ways. Details are found

in the papers of Nawaschin (1912) and his pupils, Müller (1912), Newton (1924), Taylor (1924, 1925) and others. A longitudinal splitting is evident in most of the chromosomes in the metaphase plates. It is usually most distinctly seen in the distal part of the chromosomes, and the pieces cut off by the constrictions generally appear undivided. As previously mentioned, the chromosomes differ much in size. Of the 20 present, 6 are very long, 4 are intermediate and 10 are small. The intermediate chromosomes do not look to be equal in size, 2 being much smaller than the others. In one of the plates shown in Fig. 1 the 6 long chromosomes are numbered 1–6, and the 4 of medium size 7–10. Where the distal
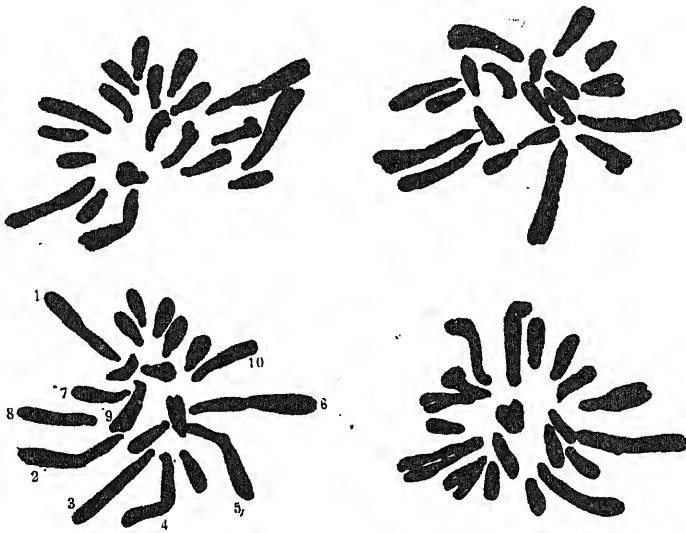

Fig. 2. Metaphase plates from root tips of *V. spiralis* ♀. (×2500.)

ends of the long chromosomes are bent upwards or downwards their full size does not show in the figures. In those plants in which differences in the size of the chromosomes have been found, there are always 2 chromosomes of the same morphological type present in the somatic divisions, and these chromosomes often exhibit a more or less pronounced pairing. This is also seen in *Vallisneria*.

The chromosomes in the corresponding divisions of female plants of *V. spiralis* at first sight look very similar to those in the male plants. This impression is fully confirmed by a closer examination (Fig. 2). Here also 20 chromosomes are present, and a special study of their size brings out facts corresponding to those in the male plants. Of the very long

chromosomes 6 are found, 4 are intermediate in length and 10 are small. Similar constrictions are found, and the longitudinal splitting takes place just as in the male plants. In Fig. 2 the chromosomes in one of the plates are numbered as in Fig. 1. It is a question of much importance whether there is any difference in the chromosome equipment of male and female plants. That the number is the same in both cases is beyond any doubt. More difficult to decide with certainty is the question of any difference in relative size and structure. So far as my observations go, and I have paid a special attention to this point, the chromosome sets in males and females are identical also in respect of their morphology. It is however impossible to be absolutely certain that a very slight difference does not exist.

In the next figure (Fig. 3) the chromosomes in root-tip divisions of the male *V. gigantea* are shown. As mentioned before, the number is 40.

Fig. 3. Metaphase plates from root tips of *V. gigantea* ♂. (×2500.)

It is of course not in every metaphase plate that this considerable number can be counted with certainty. One needs divisions in the larger cells of the root, in which the chromosomes are well spread. In such cells the counts do not leave any doubt. Also in *V. gigantea* the chromosomes are very different in size. The size classes correspond to those in *V. spiralis*, large, intermediate and small chromosomes being present. The numbers belonging to the different groups can be determined in some cases when sufficient care is taken. Often the longer chromosomes bend out of the plate, and then it is not easy to say whether the chromosome is a long or an intermediate one. One does however reach the conclusion, that 12 chromosomes are long, 8 intermediate and 20 small. The number in each size class is exactly double that of the corresponding class in *V. spiralis*. Another fact to which it is worth while calling attention is that the size of the chromosomes of corresponding types in the two

species is the same. This can be seen by comparing Figs. 1 and 2 with 3 and 4; the figures are all drawn to the same scale.

In the female plants of *V. gigantea* 40 chromosomes are also found (Fig. 4). As to the number there can hardly be any doubt. To classify all the chromosomes according to their size is more difficult. The result is however again that 12 are long, 8 intermediate and 20 small just as in the male plants. Nor, as far as one can see, is there in the case of *V. gigantea* any morphological difference in the chromosome sets of male and female plants.
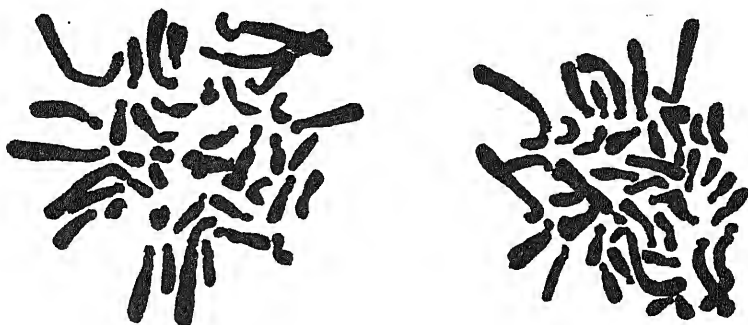

Fig. 4. Metaphase plates from root tips of *V. gigantea* ♀. (×2500.)

The material of male and female *V. spiralis* is from three different places in England and Denmark, and most likely not of the same clone. Root tips of the male *V. gigantea* have been secured from six localities in England and Denmark. The male *V. gigantea* seems to be much more commonly grown than the female, the material of which I only succeeded in getting from Hamburg after a special inquiry. There is reason to believe that living plants of this kind have been only once introduced in Europe.

Summarising the facts obtained from the investigation of the somatic divisions of *V. spiralis* ♂ and ♀ and of *V. gigantea* ♂ and ♀ we can say that the chromosomes in the two sexes of both species are identical in number and morphology, and that *V. gigantea* is a straight tetraploid in comparison with *V. spiralis*.

II. *Reduction division and pollen-grain divisions in* V. spiralis *and* V. gigantea.

Of material for studying the reduction division I have had only a little of *V. spiralis* and nothing at all of *V. gigantea*. In the material of the male plants of *V. spiralis* a series of stages was present, ranging from early heterotypic prophase to the late anaphase of the same division.

But as the sections were rather thin it has been impossible to find an untouched diakinesis, or to count with certainty the number of chromosomes in two corresponding anaphase plates. What in the slides is of significance for determining pairing and number of the chromosomes is shown in Fig. 5.
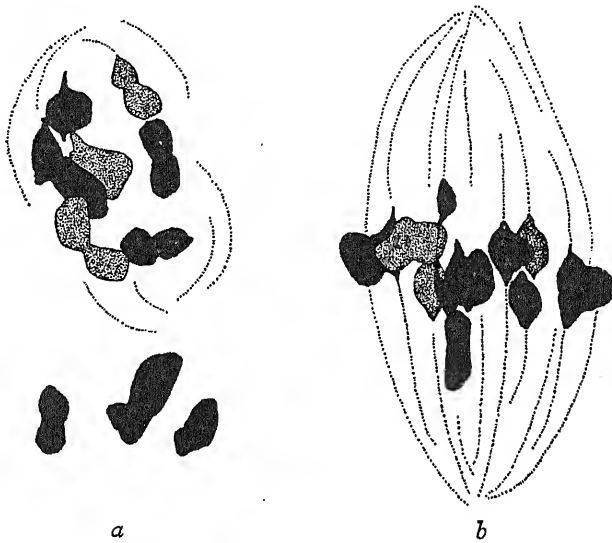

a   b

Fig. 5. Reduction division in *V. spiralis* ♂. (×2500.) (*a*) The chromosomes are moving into the plane for heterotypic metaphase; the three drawn below are from the following section. (*b*) Late heterotypic metaphase, one chromosome already divided.

The chromosomes in the first figure are just arranging themselves for the heterotypic metaphase after the nuclear membrane has dissolved. Ten gemini are found, distributed in two sections. They differ in size, 3 being large, 2 intermediate and 5 small, in accordance with the facts found in the somatic divisions. In the other figure a late heterotypic metaphase or beginning anaphase is seen. The majority of the gemini present have a rhombic shape, a few are rod-shaped, and one is in advance of the others, the parts already moving towards the poles. It is not easy in this case to arrange all the chromosomes according to their size, but what can be seen is in accordance with the other figures.

As mentioned before, Winge in his well-known paper on sex-chromosomes in plants (1923) deals with *V. spiralis*, claiming for this plant the presence of sex-chromosomes of the *XO-(Protenor)* type. The male plants are said to be heterogamous since two types of pollen grains could be demonstrated, some containing a large constricted *X*-chromosome,
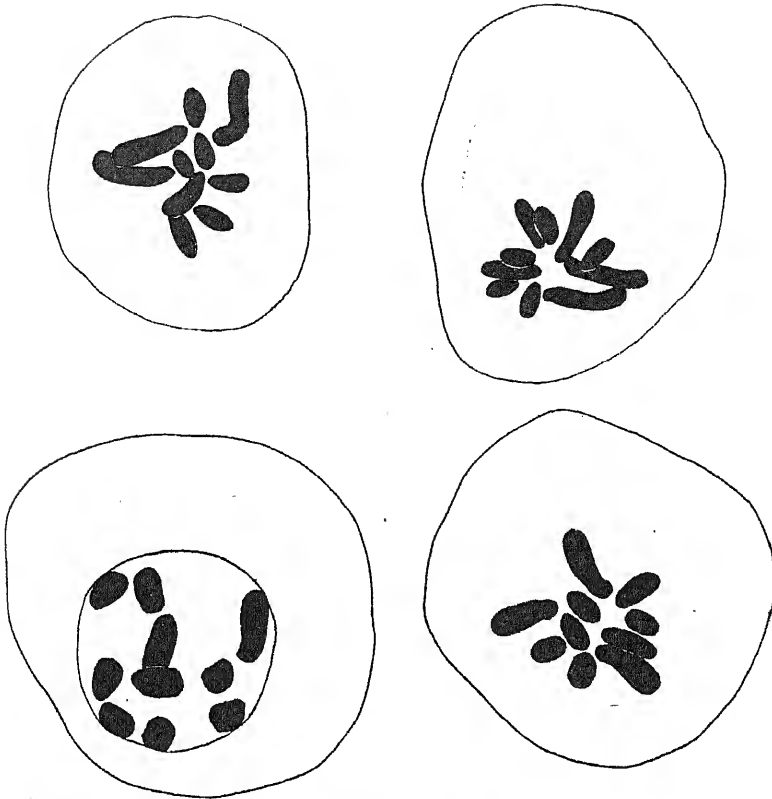
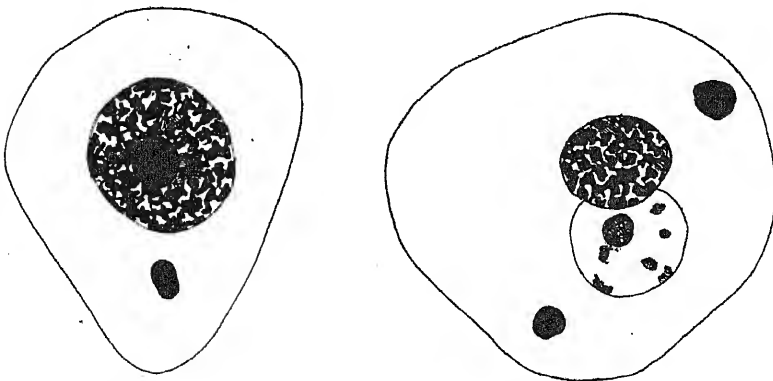Fig. 6. Division of the primary nucleus in pollen grains of *V. spiralis.* (×2500.)

Fig. 7. Young pollen grains of *V. spiralis*; note the excluded chromosomes. (×2500.)

others being without this chromosome. Winge based his view on this evidence alone, and did not determine the somatic numbers directly. It is seen at once that Winge's view is quite incompatible with the facts brought forward in this paper. In order, however, to get an explanation of this divergence I have paid some attention to the pollen-grain divisions. At my request Winge kindly placed his slides at my disposal, and also gave me the rest of his imbedded *Vallisneria* material which, together with the original slides, form the basis for the following description.

I may add at once that I can confirm the facts brought out by Winge. Pollen grains with 8 chromosomes occur together with pollen grains which besides these have a highly constricted X-chromosome. Winge says about this (1923, p. 11): "The X-chromosome is, as will be seen, divided into two, a larger portion, of the same dimensions as the largest autosomes, and a smaller answering to the smaller autosomes. At a casual glance, then, one may easily arrive at the chromosome number 10 instead of 9 in such plates." To me there is no doubt that the chromosome number in these cells (corresponding to Winge's Figs. 21, 22, 23) really is 10, and that the X-chromosome is only two autosomes, a large and a small, which happen to be lying end to end. The pollen grains in Fig. 6, with which many others are in accordance, give direct evidence for this view. The two uppermost cells are from Winge's original slides. The metaphase of the first division is shown in both, and 10 chromosomes (3 large, 2 intermediate and 5 small) lie distinctly apart. The two lower figures are from my own slides, the left showing a prophase, the right a metaphase; also here the presence of 10 chromosomes is obvious. The measurements of the chromosomes made by Winge point to the same; the larger part of the X-chromosome is exactly of the length of a large autosome ($4 \cdot 6 \mu$) and the smaller part identical with a small autosome ($2 \cdot 2 \mu$).

As has now been demonstrated, most of the divisions exhibit 10 chromosomes, and only in a few cells are 8 or 9 chromosomes found. To this may be added some cases where numbers exceeding 10 have been found. I have seen several cells with 11 chromosomes, and at least one with 12. This variation in the chromosome number in the young pollen grains, which accounts for Winge's mistake, is due to irregularities in the reduction division.

This is evident from Fig. 7. In pollen grain shown on the left one chromosome is eliminated, and as the primary nucleus has not yet divided, the elimination must have taken place in the reduction division, either the heterotypic or the homotypic one. Several grains like this

can be found in the slides. In the other pollen grain figured 2 chromosomes have dropped out. The elimination will account for the lower numbers observed; and if the chromosomes happen to be included in the nucleus again later on, numbers exceeding 10 may result; or else these may be due to non-disjunction in the reduction division. Whether the irregularities in question occur in all Vallisnerias, òr are confined to plants grown in aquaria in northern countries, nobody knows. Several factors, *e.g.* unfavourable temperatures, different chemical agencies, etc., are known to affect the reduction division and cause irregularities.

Divisions in the young pollen grains of *V. gigantea* have also been found. Some of the most distinct plates are seen in Fig. 8. The left plate is a metaphase of the first division; 20 chromosomes are present, among

Fig. 8. Divisions of the primary nucleus in pollen grains of *V. gigantea*. (*a*) Metaphase plate. (*b*) Two corresponding anaphase plates. (×2500.)

which the 6 long ones are easily picked out, the 4 intermediate ones being much less distinct. The other figure shows the corresponding anaphase plates of the same division; they are quite in accordance with the previous metaphase.

Summarising the facts regarding reduction division and pollen-grain divisions we may say that they are in full accordance with what was learned from the somatic divisions. The haploid number in *V. spiralis* is 10 (20 diploid), in *V. gigantea* 20 (40 diploid), and the chromosomes also in size and morphology correspond very well to the somatic ones. In *V. spiralis* irregularities in the reduction division are not uncommon and give rise to pollen grains with aberrant chromosome numbers.

III. *Discussion.*

From the facts given above it follows that the *XO-(Protenor)* type of sex-determination should for the present be omitted from the vegetable kingdom, although another case has been published by Meurman (1925) in *Dioscorea sinuata* Vell. The author, however, himself says (p. 90) that

his result is "not quite certain"; indeed the facts produced are so few, that it will be safer to look upon it as "quite uncertain."

In the families of *Hydrocharitaceae* and *Najadaceae,* which include the relatives of *Vallisneria,* there is no reason to believe the *XO*-type to exist. *Elodea gigantea* has been investigated by Santos (1923), who reported an *XY*-pair to be present. In the family of *Najadaceae, N. marina* L. has been the object of study for several cytologists (Guignard, 1899; Cl. Müller, 1912; Tschernoyarow, 1914). Guignard found the chromosome number in the reduction division in the pollen to be 6; Cl. Müller, studying the pairing and morphology of the chromosomes in root-tip mitoses, gave the diploid number as 14 and supposed Guignard to have missed a very small chromosome, present *in duplo* in the somatic divisions. Tschernoyarow, one of Nawaschin's pupils, working on the hypothesis of this author, who regards the trabant-chromosomes as comparable to the sex-chromosomes in insects (a hypothesis which is evidently wrong), figures somatic divisions of male and female plants of *Najas* as well as the reduction division in the pollen. In his root-tip slides the 2 very small chromosomes were always attached to 2 long ones, and therefore to be looked upon as trabants, this giving the diploid number 12, corresponding to the haploid 6, already found by Guignard and confirmed by Tschernoyarow. Concerning the number and morphology of the somatic chromosomes in the two sexes, he says (p. 412): "dass sowohl die männlichen wie die weiblichen Individuen einer vollkommen identischen Chromosomensatz besitzen, da sowohl in den einen als auch in den anderen ein Paar gleicher Trabanten vorhanden ist." The chromosomes in the two sexes are identical both as to number and shape.

The present *Vallisneria* material affords a basis for attacking the interesting problem of sex-differentiation and polyploidy. Most biologists with some knowledge of chromosome numbers and their relation in animals and plants are aware that polyploidy hardly ever occurs in the animal kingdom, but is very common in plants, and that an explanation of this is probably to be sought for in the fact that most animals are dioecious, most plants hermaphrodites (see Muller, 1925). Until lately no case of polyploidy in strictly dioecious plants was known. The Vallisnerias described here are the first example in which we must assume the doubling process to have taken place in a dioecious plant. There may be another in the genus *Elodea*; *E. canadensis* has the haploid number 12 (Wylie, 1904), *E. gigantea* 24 (Santos, 1923). But the detailed cytology of *E. canadensis* has not yet been worked out and *E. gigantea* is, judging from Santos' work, although tetraploid in number, not tetraploid

(didiploid) in its chromosome morphology, so this case is still open to discussion. Heteroploidy is also found in the dioecious species of the genus *Rumex* (Kihara and Ono, 1923; Meurman, 1925) and in the family of *Salicaceae* (Blackburn and Harrison, 1924). But many hermaphrodite *Rumex* species exist, and in the case of the *Salicaceae* male organs are not uncommon on the female plants.

From a doubling of the chromosome number in hermaphrodite plants there will arise a tetraploid hermaphrodite, which has from the very start corresponding male and female gametes. In dioecious plants a doubling in the male is likely to produce a tetraploid male, whereas the same process in a female will produce a tetraploid female. On these plants gametes of one sort only are present, and these are of no use when the corresponding ones are missing. The doubling process in dioecious organisms is therefore not likely to become of permanent value, the tetraploid males or females usually dying out without giving rise to tetraploid offspring. These can only be produced in the very rare cases, where the doubling process happens at the same time in a male and a female individual growing in the same locality.
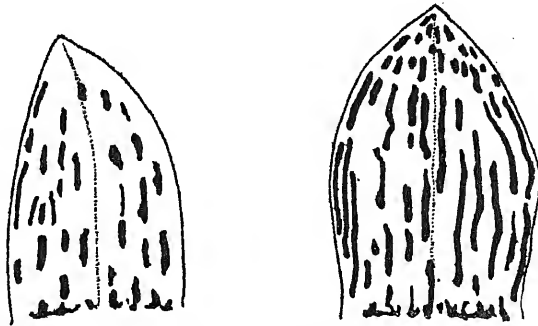
Fig. 9. Sepals of *V. spiralis* (left) and *V. gigantea* (right), showing blackish stripes. (×35.)

In the Vallisnerias there is reason to believe that the tetraploid *V. gigantea* has been formed by a doubling of the chromosome number in *V. spiralis* or a closely related type. The two species are distinctly different, but the differences all seem to me of the kind for which a mere doubling of the chromosomes will account. From Graebner's description (1913), with which the material I have seen (living plants and type specimens from Bureau of Science Herb. No. 11936) is in conformity, the *gigantea* type has the following characteristics: bigger than *spiralis* in all parts, broader and 7-veined leaves, stolons not abundant; the

stigmas rather short and narrow and their tips a little corniform; spatha, fruit wall and sepals with narrow blackish stripes. The differences how-ever all have a quantitative character for which the tetraploidy well accounts. The blackish stripes, for example, are also present in *V. spiralis,* but are shorter and less numerous (Fig. 9).

It is a well-known fact that a multiplication of the chromosome number occasionally occurs in plants. Several cases have been found by chance, and Winkler (1916) has even produced tetraploids experimentally. That such doubling of the chromosome number also takes place in *Vallisneria* is evident from Fig. 10 in which are shown (*a*) the nuclei of two

a   *b*
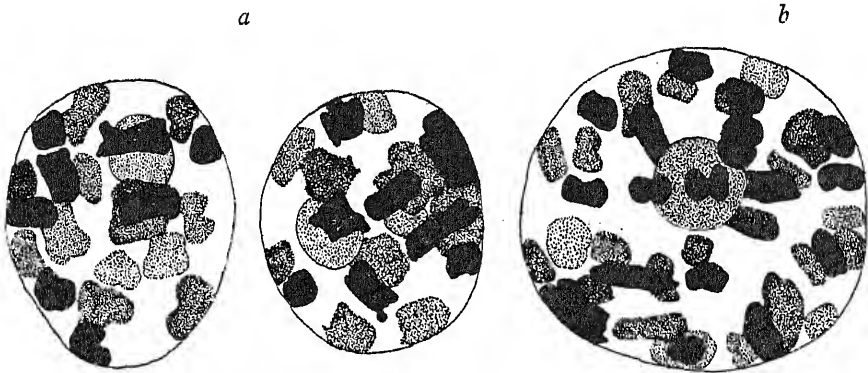
Fig. 10. Nuclei of tetraploid (*a*) and octoploid (*b*) pollen mother cells in *V. spiralis.* (×2500.)

tetraploid pollen mother cells found in one of my slides, and (*b*) an octoploid nucleus found in another slide. Owing to the indistinctness of the gemini in diakinesis it has been impossible to determine the actual number present in the cells, but it is about 20 in the former and 40 in the octoploid. Such doubling of the chromosome number, occurring of course in the somatic tissue, may give rise to tetraploid plants; and it is my opinion that *V. gigantea* has been formed in this way, the males from a male diploid and the females from a female diploid, although it is not excluded that one of the sexes may have arisen as a somatic mutation from the other.

The Vallisnerias have an almost unlimited power of vegetative propagation. This has made the formation of a tetraploid species possible in these dioecious plants, because it means that a male *V. gigantea* for example may exist for centuries, waiting for females to be formed. The description by Graebner (1913) only mentions female plants, and possibly

males were not growing in the locality in the Philippine Islands where the type specimens were found. It has not been possible to trace the history of the male *V. gigantea* grown in Europe.

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BIZZARRIA—A CLEAR CASE OF PERICLINAL CHIMERA.

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(Contribution No. 10.)

(With Three Plates.)

THE bizzarria orange is generally referred to as the typical illustration of a sectorial chimera. Early accounts of Nati[15] and others following him accredit its origin to a defective grafting between citron (*Citrus medica* Linn.) and sour orange (*Citrus Aurantium* Linn.), accidentally obtained by a Florentine gardener in 1644. An illustration of Risso and Poiteau[21], later copied by Engler and Prantl[9], strongly suggests a sectorial character. Fuller accounts of its origin and criticism of its nature were given by Gallesio[10], Darwin[8], and Penzig[17], [18], but time was premature for these authors to arrive at any satisfactory conclusion. Strasburger[23], discussing from his cytological study of the vegetative sphere of citron, sour orange and bizzarria, concluded that the bizzarria cannot be a case of somatic hybrid but possibly is a sexual hybrid, though there is no proof of it on record. Winkler's[28] discovery of plant chimeras caused by grafting, eventually made Strasburger change his opinion, and he[24] suggested the possibility of the existence of "hyperchimera" in plants, presenting bizzarria as a possible case of it. Baur[2], from his studies on the variegated *Pelargonium*, advanced the opinion that plant chimeras are divisible into two classes, sectorial and periclinal. Winkler[29], and Buder[5], using the terminology of Baur, classed all of the tomato-nightshade graft-hybrids as periclinal chimeras, with the exception of *Solanum Darwinianum*, which Winkler classed as "Verschmelzungs-Pfropfbastard." Neither Winkler nor Baur made mention of bizzarria, but since Baur[3] maintains that hyperchimera is in anatomical construction simply a periclinal chimera, bizzarria, according to his definition, may be classed as a periclinal chimera. This deduction, however, failed to attract the attention of succeeding writers.

Goldschmidt[11] was probably the first author to cite bizzarria as an undoubted case of sectorial chimera, and the unfortunate coincidence of Haecker's book[12] appearing almost at the same time created

a wide acceptance for this assumption. Together with the misleading Risso-Engler illustration of bizzarria, many textbooks copied the inference as an example of the rather rare occurrence of sectorial chimera in the plant kingdom. Later writers, as in the cases of Plate (19), Molisch (14), and Babcock and Clausen (1), seem to tend to recognize the partial occurrence of periclinal formation of the fruit, but no critical statement is presented. Careful reading of the anatomical account given by Strasburger (23) will show that the occurrence of sectors of both citron and sour orange flesh within a single fruit is exceptional, and his statement is too unconvincing to warrant the acceptance it received. Risso and Poiteau (21) state that the citron character does not affect the sour orange centre, or penetrate as far as its axis, but their painting shows that the bizzarre outline does not affect the citron centre. An exactly similar nature of the pulp is later exhibited by the drawing of Poiteau and Turpin (20).

In the winter of 1922–1923, the writer made a critical study of the bizzarria plants at Florence and La Mortola, through the courtesy of the Botanical Institute of the University of Florence and the Giardino Hanbury of La Mortola near Ventimiglia, Italy. Some potted plants in the greenhouse of the former, labelled bizzarria, were found to be normal sour orange, while some others bore binary oranges. None was the typical bizzarria fruit as so excellently illustrated by Poiteau and Turpin (20). In the field of La Mortola garden, a plant presented a very typical bizzarria, bearing a fine bifacial fruit which was later submitted to the examination of the writer. This particular fruit is described below (Plate VI).

Fruit medium in size, 19·6 cm. in girth, 6·3 × 6·1 cm. in diameter, and 5·8 cm. high, longitudinally divided into $\frac{1}{2}$ part sour orange and $\frac{1}{2}$ part citron. Sour orange part oblate, about 5·5 cm. across and 4·2 cm. high, apex rounded, slightly depressed toward the stylar point, base much depressed to the calyx; surface even, indistinctly very fine streaked, finely pitted with rather indistinct oil cell dots; colour green, beginning to change into orange; the peel breaking longitudinally at one point, disclosing more or less sunken citron-like surface, the striations becoming more distinct at the stylar end. Citron part very rough, irregularly tuberose, longitudinally streaked, striations irregular, branching, deeper coloured, showing at one place clearly the texture and colour of sour orange; colour of peel pinard yellow to light cadmium (Ridgway, *Color Standard*, Plate IV), oil cell dots far apart, size uniform, shallowly concave, distribution irregular. Calyx flat, very irregularly lobed, oil cell dots indistinct but projecting like warts; texture thick, glabrous.

Section periclinal, normal citron pulp enclosed in a sour orange peel, the latter being partly penetrated by citron character. Sour orange rind thick, 9–11 mm., coloured, with imbedded fibres; oil cells close, fine, oblong, homogeneous in size but arranged in irregular rows; inner layer containing several fissures forming empty holes. Citron rind distinctly separate from the former, connecting at a certain place with the pulp ball, pure white, about 20 mm. thick, texture solid, no imbedded fibres, oil cells large, oblong, distant, outline not definite, arranged in different depths, some reaching far interior, very light greenish yellow. Pulp ball small, round; segments 11, regular; outer end rounded, inner end obtuse; central column large, 10×6 mm., solid, fibre strands circinate, not prominent; segment wall thick, holding white pith between. Pulp white, solid, transparent, acid, vesicles elongated, parallel, very few in number, flexible. Seeds few, small, plump, smooth, rosy outside, testa thin, tegmen beautiful rosy purple, containing white mono-embryo.

The La Mortola plant is a dwarf tree about one metre in height with a single main trunk inclining toward north-east. The plant bears three distinct kinds of foliage; namely, typical sour orange leaves, typical citron leaves and typical bizzarria leaves as painted by Risso and Poiteau [21]. The sour orange leaves are characterized by a thick, deep green blade and definitely winged petiole. The citron leaves are oblong, lighter coloured, much thinner, distinctly serrate on margin, and devoid of petiole wing. The bizzarria leaves in general resemble the sour orange leaves, but are quite irregular in outline and variable in shape. They are occasionally very much narrowed and boat-shaped, and are sometimes more or less variegated. An abnormal linear form markedly tapering at both ends is also found. The petiole wing is quite variable, ranging from linear to oboval. The first horizontal branch of the La Mortola plant comes out quite near to the ground, stretching toward the north-east, and bears typical bizzarria shoot at the terminal (Plate IV, C). A side branch, coming upward about the middle of this branch, shows typical citron leaves at the tip (Plate IV, A). The second branch, starting from the trunk about 7 cm. above the ground, rises southward with rather strong inclination, 67 cm. in total length. Two fruits are borne on this branch, the terminal one being the one described above. The second fruit, not taken for study, is also bizzarria, appearing like an immature warty citron with deep green sour orange streaks. The foliage below these fruits presents normal bizzarria characteristics. Nearly all of the side branches of this second branch have bizzarria characteristics, except two small citron shoots rising about the middle of the second side branch, and the

sour orange terminal shoots of the first side branch. The latter is a very remarkable case of the segregation of very stable sour orange from rather variable bizzarria, the demarcation being so clear that it looks as if two different kinds of shoots were grafted together. The bizzarria foliage below the joint is particularly small, narrow and acutely tapering, while sour orange leaves attached are large, oval, well spreading and widely alate. The writer [25], [26], [27] has been studying many remarkable cases of bud variation and vegetative reversion between "Wase" (*praecox*) and normal characters in Satsuma orange (*Citrus unshiu* Hort.), but he has never observed such remarkable segregation on a single growing axis.

From the joint of this second branch, the main trunk rises obliquely for a distance of 14 cm. with inclination toward the north-east, and there forks into two large spreading branches, leaving a slender straight shoot ascending in the opposite direction from the main trunk. The forking branches again fork almost alike on the two sides and are all typical sour orange, none bearing bizzarria foliage, while the slender one is bizzarria throughout. In this case segregation must have taken place at the point where the trunk made the first forking. The specimen of sour orange leaves (Plate IV, B) was taken from the second branchlet from the north. The plant as a whole is a bizzarria with large fan-shaped top of segregated sour orange, and with occasional reversion to both sour orange and citron. The tree is trifacial in nature just exactly like *Crataegomespilus Dardarii*, a plant of which was studied by the writer with the kind aid of Prof. W. Johannsen of the University of Copenhagen. This Bronvaux medlar regularly sends forth two kinds of reverted shoots, *i.e.* pure medlar and pure whitethorn, so that three kinds of branches are present on the same tree, just as in the case of the bizzarria orange here described. According to Meyer [13], *Crataegomespilus Dardarii* is a periclinal chimera, with the upper two layers of *Mespilus* over the core of *Crataegus*[1]. Here the *Crataegus* core occasionally penetrates the *Mespilus* mantle, or it drops from the covering, leaving pure *Mespilus*. Similarly, the sour

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[1] Since the above was written Prof. Punnett kindly called the attention of the writer to the peculiar nature of the *Crataegomespili* studied by Prof. F. E. Weiss, showing intermediate characteristics of both graft parents. Prof. Weiss (in *Mem. and Proc. Manchester Lit. and Phil. Soc.* LXIX. 1925, No. 9, pp. 1–6) boldly suggests that these graft-chimeras are somewhat like seed-hybrids, showing segregation of parental characters in their somatic tissues. The writer, however, believes that plant chimeras are not necessarily very strictly dual in nature, and it is possible that certain characters may be influenced by others through close contact of widely different elements. In the case of the epidermal tissue of *Crataegomespili*, the outline of the cells must be influenced by the underlying inside element, while other essential morphological characters (like hairiness, cell thickness and contents, etc.) are left quite intact.

orange mantle may be penetrated by the citron core; or the citron core may entirely drop out and the pure sour orange branch results.

The first plant studied at Florence also possessed three distinct types of foliage (Plate V). Very small immature citron fruit was taken for study from a citron branch of this tree (Plate V, D), and a yellow streaked sour orange was taken from a bizzarria branch of the same tree (Plate V, A). The latter has the appearance of common sour orange, except for citron-coloured longitudinal striations, slightly sunken from the sour orange surface. By halving the fruit, this was found to be a bizzarria, with normal sour orange covering over a pure citron pulp ball. The protuberance of the citron peel, as found in the previous specimen, does not seem to be an invariable characteristic, but, as noted above, the presence of fissures in the sour orange peel perhaps enables the enclosed citron element to push out readily to form an outgrowth. It is also quite possible that fruit with pure sour orange cover and citron core may be found. No fruit with sour orange appearance was borne on this tree, though the sour orange leaves were present (Plate V, C). All sour orange fruit examined from the second plant showed typical sour orange characteristics throughout the centre. This tree, originally propagated from a bizzarria plant, seems to have reverted completely to a sour orange, and the citron element is entirely lost from the foliage and fruit.

The typical bizzarria fruit described before, that from La Mortola, presents sufficient evidence that bizzarria is a periclinal chimera, the external cover of sour orange having no influence on the citron core. The cover is fundamentally sour orange and the penetration of the citron element from the citron carpel is made easy by the weaker construction of the outer layer. Fine striations longitudinally running on the fruit surface may have some significance in this connection. Close examination will at once prove that the citron skin is not a pure citron, but is longitudinally streaked with a substance corresponding to that of sour orange. It looks as if the citron substance is forced out through the matrix of sour orange, leaving the linear patches of the ground substance in the form of parallel striations. In transversely halving the fruit, this point is most clearly demonstrated by the presence of distinct sour orange peel elements left in the citron portion (Plate VI). The texture of citron peel is highly differentiated from the sour orange substance, expanding outward in a fan-shaped mass quite narrowed at the inner portion reaching as far as the carpel. The segment enclosed is in every respect a citron, and no Citrus fruit is known with such light-coloured pulp and mild acid flavour. Although Strasburger(23) mentions that bizzarria is sterile, the

writer found seeds of thoroughly citron character. The purplish-rosy colour of the tegmen is not to be found in any other kind of Citrus besides citron.

Critical study of this much discussed graft-hybrid thus brings us to the conclusion that this is a clear case of periclinal chimera, a citron core covered by sour orange coating. The citron peel, suggestive of being a sectorial chimera, is merely the penetration of citron element through the covering, just as the pure citron shoot comes out from a bizzarria branch. A pure sour orange shoot is similarly formed by replacing the citron element of the central portion, and in the same way the sour orange sector may be present among the segment of fruit partly replacing the citron carpels. The sectorial formation of the pulp occasionally mentioned is explained by such a partial replacement of the internal element by the outside substance. The trifacial nature of graft-hybrids is only explained by such replacement of antagonistic elements through invasion both outward and inward. Graft-hybrid chimeras are unstable in their nature; the union between the outside and inside elements can readily be broken, the single element occupying the whole tissue wherever there is a chance, because this condition is more stable than the original dual formation. This reversion to the original parent seems to be analogous to the vegetative reversion associated with bud variation, but the latter case is better explained by reversible allelomorphic transformation or by chromosomal mutation, rather than the chimeral construction of the original bud-mutant. Cavara[6] stated that sectorial chimera occurs in a canaliculate sour orange found in the Naples Botanic Garden, but the writer's observation proved that this is a case of vegetative reversion developing sectorially from the original canaliculate bud-mutant, and such cases are very common in other bud-mutants found in the Citrus fruits, as is previously shown by the writer[25]. Cramer[7] cites cases where a spontaneous orange sector is found in the fruit of common lemon, and Oudemans[16] gives a fine illustration of a halved lemon containing sweet orange segments. Savastano and Parrozzani[22] also give striking cases of such sectorial fruits, and an orange-streaked lemon illustrated by Babcock and Clausen[1] is explained as a chimera. Such spontaneous occurrences of orange-coloured sectors in light-coloured Citrus fruit are probably the emergence of the hidden element come up from the sour orange stock, and are not to be ascribed to bud mutation or to typical chimera as seen here in bizzarria orange. Imperfect union between the scion and the stock may carry up a patch of the stock tissue surrounded by a majority of the scion tissues. The existence of such "island" of

other element is described by Bateson(1a) in a zonal pelargonium. Such may be termed hyperchimera in Strasburger's sense, but is naturally a periclinal, either affecting the core or rind. The occurrence of periclinal chimera originating perhaps as graft-hybrid, is not very rare in the Citrus fruits. Darwin(8), and later Bonavia(4), summarize two distinct cases of such, one from Smyrna and the other from Alexandria. Lemon (*Citrus Limon* Burm.) and sweet orange (*Citrus sinensis* Osbeck) sectors are found in the same fruit, just like bizzarria. The writer witnessed such a dual plant in Alabama in 1918, a satsuma orange graft with a trifoliate orange sector rising from the stock. The plant was a nursery tree of about three years old, but the writer never saw the fruit.

Judging from the material here presented, the writer inclines to the opinion that periclinal construction occurs with greater frequency than true sectorial formation throughout the epidermal and cortical tissues. Sectorial chimera, as encountered in the plant kingdom, may be mostly a temporary feature of constitutional periclinal chimera, so explained merely because its true nature had been insufficiently analysed.

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EXPLANATION OF PLATES IV—VI.

PLATE IV.

Branches of bizzarria, sour orange and citron, all taken from a tree planted at Giardino Hanbury, La Mortola, near Ventimiglia, Italy.

A. A bizzarria branch terminating with pure citron foliage.

B. A branch of thoroughly sour orange character from the same tree.

C. A bizzarria branch with typical narrowed leaves.
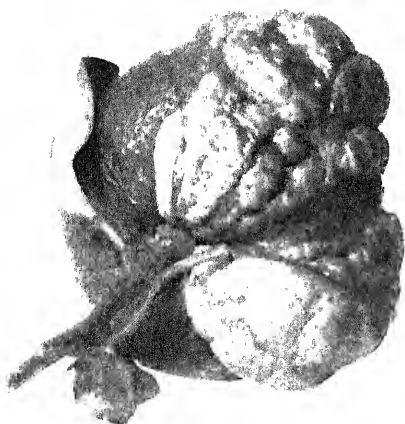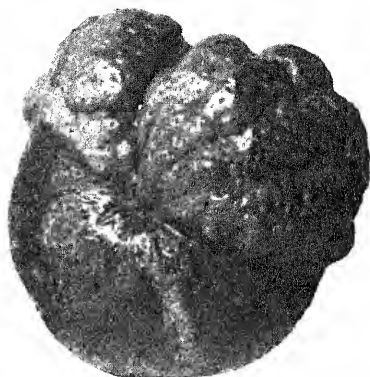
PLATE V.

Three kinds of branches collected from a potted tree of bizzarria, from the greenhouse of the Botanical Institute, University of Florence, Italy.

A. A bizzarria branch with a striped fruit (bizzarria, but citron sectors forming sunken striations without projecting in protuberance).

B. A bizzarria branch, showing variability of petiole wing.

C. A pure sour orange branch without fruit.

D. A citron branch with a miniature citron fruit.

E. A branch of bizzarria, partly segregating pure citron leaves (upper two).

PLATE VI.

A fruit of bizzarria collected from a bizzarria branch of La Mortola plant.

Upper line. Cross-section and apical view. Note the clear demarcation of sour orange and citron rind, and light coloured pure citron pulp.

Lower left. Basal view of the same.

Lower right. Side view of the same. (Note very typical narrow, boat-shaped leaves.) Size slightly reduced. (Tanaka and Hosomi photo.)

THE INHERITANCE OF BLACK, YELLOW AND TORTOISESHELL COAT-COLOUR IN CATS.

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THE problem of the inheritance of black, yellow and tortoiseshell coat-colour in cats is both complicated and interesting. Comparatively few carefully controlled experiments have been carried out, but considerable data have been collected from breeders, and certain facts have emerged quite clearly. Normally the cross black ♀ × yellow ♂ gives tortoiseshell ♀♀ and black ♂♂: the reciprocal cross, yellow ♀ × black ♂, gives tortoiseshell ♀♀ and yellow ♂♂. Tortoiseshell males occur only as very rare exceptions.

These observed facts have been explained in many different ways.

Doncaster (1904) suggested that yellow is completely dominant over black in the male, incompletely dominant in the female, so giving tortoiseshell daughters and yellow sons from the cross yellow ♀ × black ♂. Little (1912) pointed out, however, that the reciprocal cross black ♀ × yellow ♂ gives *black* sons and tortoiseshell daughters, and that therefore dominance of yellow over black in the male is not an adequate explanation of the observed facts. He suggested that both yellow and black are sex-linked, a black ♀ being **BB**, a black ♂ **B–**, a yellow ♀ **YY**, and a yellow ♂ **Y–**. In the same year Doncaster (1912) concluded that yellow *only* is sex-linked, and that black is present in all gametes, yellow being completely dominant over all black in the male, incompletely dominant in the female. In 1913 Johannsen put forward a theory almost exactly like that offered by Doncaster in 1904, except that he looked upon black as being dominant to yellow in the male, whereas Doncaster supposed yellow to be dominant to black. In 1916 Ibsen suggested that tortoiseshell is due to a separate factor, **T**, which can only act in the presence of black, **B**, causing it to be restricted to spots with yellow areas between. He considered black, **B**, to be dominant to yellow, **b**, and suggested that normally **T** is closely linked with **b**. He supposed both pairs of allelomorphs, **Tt** and **Bb**, to be sex-linked. Wright in 1918, discussing the chemistry of coat-colour, adopted Little's idea of sex-linkage of both

black and yellow, but evidently considered yellow to be simply absence of black. In 1919 Little modified his earlier hypothesis to the extent of placing **B**, black, in *all* X-chromosomes and making **Y**, yellow, and **y**, the absence of yellow, a separate pair of sex-linked allelomorphs. He suggested that one dose of yellow is completely epistatic to one dose of black giving yellow in both sexes, but that one dose of yellow to two doses of black gives tortoiseshell.

Apart from Doncaster's first suggestion (1904) and the similar one put forward by Johannsen in 1913, any one of the above hypotheses will account satisfactorily for the observed normal facts of inheritance of black, yellow and tortoiseshell.

There are, however, a number of exceptional facts of inheritance. A tortoiseshell male does occasionally appear, and somewhat more frequently black daughters are produced from yellow sires. There are also one or two cases on record of a yellow female having bred as a tortoiseshell. Many different explanations, based on one or other of the above hypotheses, have been put forward to account for these exceptional individuals, but no theory so far offered has been able to bear the full weight of the recorded facts, and the whole problem of the inheritance of black, yellow and tortoiseshell remains unsettled. (A detailed discussion of the rival theories is given by Bamber in *Bibliographia Genetica*, Vol. III. pp. 14–44.)

Either yellow or black or both are certainly sex-linked, but the ordinary facts of inheritance can be explained equally well by assuming:

(1) that black and yellow are both sex-linked and are allelomorphs, *i.e.* two positive factors which are alternative to one another;

or

(2) that both are sex-linked but are not allelomorphs, black being present in all X-chromosomes, one dose of yellow being completely epistatic to one of black;

or

(3) that both are sex-linked but are not allelomorphs, yellow being present in all X-chromosomes, one dose of black being completely epistatic to one of yellow;

or

(4) that yellow only is sex-linked and that black is present in *all gametes* (*i.e.* in an autosome), one dose of yellow being completely epistatic to *all* black in the male, incompletely so in the female;

or

(5) that black only is sex-linked and that yellow is present in *all*

gametes, one dose of black being completely epistatic to *all* yellow in the male, incompletely so in the female.

Two of the above possibilities (3 and 5) are, however, usually discounted because at least three matings between yellow cats have been definitely recorded as giving black or tortoiseshell in the offspring (Doncaster, 1913; Whiting, 1918), whereas there is only one doubtful record of two blacks ever having given tortoiseshell in the offspring (Bonhote, 1915).

Notwithstanding the conflicting evidence it seems certain that, whatever may be the exact relationship between black and yellow, either there is a difference in dominance in the two sexes or both colours are sex-linked.

The observed results of carefully controlled experiments are few. Little (1912) and Whiting (1915, 1918, 1919) have both recorded results from their own experiments but these involve comparatively few matings. Doncaster (1913) has given the fullest account of the inheritance of black, yellow and tortoiseshell but his data were collected from breeders for the Cat Fancy and were not the results of carefully controlled scientific experiments. Bonhote (1915) also gives many facts, but again the majority were obtained from breeders, and most of these facts had already been given to Doncaster and were included in his 1913 paper; they are, therefore, no additional evidence.

Thus there is not a very firm foundation of fact on which to erect such a weighty superstructure of hypothesis.

In the hope of throwing some additional light on the subject we have been carrying out experiments with cats during the past four years. The experiments have been chiefly genetical but a little physiological work has also been included. One of us suggested, Bamber 1922, that, if there is a difference of dominance between black and yellow in the two sexes, it might be possible to alter the colour of a male by turning his physiology towards the female condition. In an attempt to do this, in 1923, three newly born yellow male kittens were castrated, and, from the time they were about four months old, two of these were fed with ovarian extract for a period of six months. The results were entirely negative. We intended to perform the same experiments with black males, but, before this was attempted, our genetical work practically proved that there is no difference in dominance in the two sexes, and our physiological work was therefore discontinued.

In reporting our breeding experiments, it is important to state clearly that our animals have been kept under conditions which preclude

the possibility of any inaccuracy in our records. The females have lived and bred in separate cat-houses with enclosed wired-in grass runs, and have not *at any time* been allowed liberty. The males have been free to roam the neighbourhood[1], but have each been confined in a separate pen with a single female at the times of mating. Our results, as recorded in this paper, are, therefore, entirely reliable.

Many different crosses have been made, and in each case there have been several litters. The total results of these experiments are as follows:

(*a*) Black ♂ × Black ♀—5 Black ♂♂; 8 Black ♀♀.

(*b*) Black ♂ × Yellow ♀—3 Yellow ♂♂; 3 Tortoiseshell ♀♀.

(*c*) Black ♂ × Tortoiseshell ♀—11 Black ♂♂; 8 Yellow ♂♂; 9 Black ♀♀; 8 Tortoiseshell ♀♀.

(*d*) Yellow ♂ × Yellow ♀—see (*g*) below.

(*e*) Yellow ♂ × Black ♀—7 Black ♂♂; 2 Tortoiseshell ♀♀.

(*f*) Yellow ♂ × Tortoiseshell ♀—1 Black ♂; 5 Yellow ♂♂; 4 Yellow ♀♀; 1 Tortoiseshell ♀; 1 Anomalous Yellow ♀ (4 *d*)[2].

(*g*) Yellow ♂ × Anomalous Yellow ♀ (4 *d*)[2]—3 Yellow ♂♂; 2 Yellow ♀♀; 1 Anomalous Yellow ♂ (Tortoiseshell?)[2]

These results are given in detail in Table I. It seems advisable to do this both because the amount of data from controlled experiments is small and because, so often, in condensing results, facts disappear which would be of value to other workers approaching the problem from a different view-point. Also, in such a controversial subject, any fact may prove to have an unsuspected significance.

Reference numbers are given in the table for all animals which have been used for breeding, so that the interrelationship of our stock can be easily followed. Arabic numerals denote individual cats; Roman numerals denote matings.

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[1] There is, however, no question as to the identity of the tom-cats. They were treated as personal friends and, as such, were readily recognisable from other cats which visited the cattery from time to time.

[2] The ♀ was yellow with a minute amount of black on the back of her right hind foot, and might therefore be called tortoiseshell. She is here classified as yellow because her breeding behaviour suggests that her yellow and black do not segregate as they do in a normal tortoiseshell; she appears to be homozygous for yellow in addition to having the small amount of black. Her son, the "anomalous yellow" male kitten had about twice as much black as his mother, but his type of colouration is so obviously the same as hers that it would be absurd to classify them differently. The whole subject is discussed in detail on p. 92.

TABLE I.

No. of Mating	Nature of mating	Black ♂	Black ♀	Yellow ♂	Yellow ♀	Tortoise- ♂	Tortoise- ♀	Anom. yellow ♂	Anom. yellow ♀
I.	♂ 3 Black × ♀ 2 Black	1	2	.	.	.	.	.	.
II.	,, × ,,	3	1	.	.	.	.	.	.
III.	♂ 1 a Black (ex XV) × ♀ 2 Black	1	3	.	.	.	.	.	.
IV.	♂ 1 a Black (ex XV) × ♀ 16 b Black (ex II)	.	2	.	.	.	.	.	.
V.	♂ 1 a Black (ex XV) × ♀ 2 a Yellow (ex XVIII)	.	.	3	.	.	2	.	.
VI.	,, × ,,	.	.	.	.	.	1	.	.
VII.	♂ 1 a Black (ex XV) × ♀ 4 Tortoiseshell	2	.	.	.	.	1	.	.
VIII.	,, × ,,	1	.	2	.	.	1	.	.
IX.	,, × ,,	.	1	.	.	.	1	.	.
X.	♂ 1 a Black (ex XV) × ♀ 5 Tortoiseshell	1	.	2	.	.	2	.	.
XI.	,, × ,,	3	1	1	.	.	.	.	.
XII.	,, × ,,	.	2	1	.	.	1	.	.
XIII.	♂ 7 Black × ♀ 5 Tortoiseshell	2	3	1	.	.	1	.	.
XIV.	♂ 13 Black × ♀ 5 Tortoiseshell	2	2	1	.	.	1	.	.
XV.	♂ 1 Yellow × ♀ 2 Black	5	.	.	.	.	.	.	.
XVI.	,, × ,,	2	.	.	.	.	1	.	.
XVII.	♂ 12 Yellow × ♀ 16 b Black (ex II)	.	.	.	.	.	1	.	.
XVIII.	♂ 1 Yellow × ♀ 5 Tortoiseshell	.	.	3	1	.	.	.	.
XIX.	♂ 1 Yellow × ♀ 4 Tortoiseshell	1	.	1	1*	.	.	.	1
XX.	♂ 12 Yellow × ♀ 4 Tortoiseshell	.	.	1	2	.	1	.	.
XXI.	♂ 1 Yellow × ♀ 4 d Anomalous Yellow (ex XIX)	.	.	1	1	.	.	1	.
XXII.	♂ 9 Yellow × ♀ 4 d Anomalous Yellow (ex XIX)	.	.	2	1	.	.	.	.

* This kitten was recorded as a ♀, but as it died young and was not dissected its sex is not absolutely certain.

TABLE I.

No. of Mating	Nature of mating	Black		Yellow		Tortoise-		Anom. yellow	
		♂	♀	♂	♀	♂	♀	♂	♀
I.	♂ 3 Black × ♀ 2 Black	1	2	.	.	.	.	.	.
II.	,, × ,,	3	1	.	.	.	.	.	.
III.	♂ 1 a Black (ex XV) × ♀ 2 Black	1	3	.	.	.	.	.	.
IV.	♂ 1 a Black (ex XV) × ♀ 16 b Black (ex II)	.	2	.	.	.	.	.	.
V.	♂ 1 a Black (ex XV) × ♀ 2 a Yellow (ex XVIII)	.	.	3	.	.	2	.	.
VI.	,, × ,,	.	.	.	.	.	1	.	.
VII.	♂ 1 a Black (ex XV) × ♀ 4 Tortoiseshell	2	.	.	.	.	1	.	.
VIII.	,, ,,	1	.	2	.	.	1	.	.
IX.	,, × ,,	.	1	.	.	.	1	.	.
X.	♂ 1 a Black (ex XV) × ♀ 5 Tortoiseshell	1	.	2	.	.	2	.	.
XI.	,, × ,,	3	1	1	.	.	.	.	.
XII.	,, × ,,	.	2	1	.	.	1	.	.
XIII.	♂ 7 Black × ♀ 5 Tortoiseshell	2	3	1	.	.	1	.	.
XIV.	♂ 13 Black × ♀ 5 Tortoiseshell	2	2	1	.	.	1	.	.
XV.	♂ 1 Yellow × ♀ 2 Black	5	.	.	.	.	.	.	.
XVI.	,, × ,,	2	.	.	.	.	1	.	.
XVII.	♂ 12 Yellow × ♀ 16 b Black (ex II)	.	.	.	.	.	1	.	.
XVIII.	♂ 1 Yellow × ♀ 5 Tortoiseshell	.	.	3	1	.	.	.	.
XIX.	♂ 1 Yellow × ♀ 4 Tortoiseshell	1	.	1	1*	.	.	.	1*
XX.	♂ 12 Yellow × ♀ 4 Tortoiseshell	.	.	1	2	.	1	.	8
XXI.	♂ 1 Yellow × ♀ 4 d Anomalous Yellow (ex XIX)	.	.	1	1	.	.	1	.
XXII.	♂ 9 Yellow × ♀ 4 d Anomalous Yellow (ex XIX)	.	.	2	1	.	.	.	.

* This kitten was recorded as a ♀, but as it died young and was not dissected its sex is not absolutely certain.

On the whole our results are in harmony with the findings of other workers, but some new facts have come to light which give rise to entirely new points of interest. These facts are:

(1) A yellow ♀ (4 d) has appeared with a minute amount of black-spotting on the back of one foot and this black seems not to have segregated from yellow in her germ cells—it was apparently transmitted *together with yellow* to one of her sons.

(2) Twenty-six yellow cats have been examined and, with one possible exception, all have been found to have a few scattered black hairs. An equally careful examination of forty-three black cats revealed only one single yellow hair.

The yellow ♀ (4 d) was at first thought to be an ordinary yellow kitten, but when she was about four months old three minute black spots were discovered on the back of her right hind foot. She was then, of course, recorded as a tortoiseshell. Naturally she reminded one of the two yellow females, recorded by Doncaster (1913) and Whiting (1918), which bred as tortoiseshells, and she seemed to support the suggestion made by Whiting (1915) that tortoiseshell females may vary from solid black to solid yellow. Whiting (1915) and Doncaster (1913) have both recorded Maltese ("blue" = dilute black) cats with only a few cream

hairs. Whiting states that his tortoiseshell females could be arranged in a series leading from one predominantly yellow to one almost pure Maltese, and our almost entirely yellow female, together with the two entirely yellow ones referred to above, which bred as tortoiseshells, seemed to complete the yellow end of the series. To account for such a series Whiting (1915) put forward the suggestion that, in addition to the colour factors involved in the production of tortoiseshell, there are also modifying factors governing the relative amounts of the two colours and so producing continuous variation from yellow to black. It was, therefore, of interest to test the mode of inheritance of the yellow and black in our anomalous yellow female (4 *d*). She herself was the offspring of a yellow male (1) and a dark mingled tortoiseshell female (4). When mature she was mated with her own father (4 *d* × 1) and produced a yellow male, a yellow female and an "anomalous yellow" male. This male was extremely interesting in that his *type* of marking was clearly the same as his mother's. On casual observation he might have been mistaken for a yellow. He had, however, more black than his mother and its distribution was different: he had a distinct spot over the left eye, a very small spot above each ear, a scarcely visible one on the right shoulder, a small one on the left side of the tail about half way down, and a minute one on the right side of the tail nearer the tip, in addition to numerous scattered black hairs throughout the yellow parts of his coat. This small amount of black-spotting seems almost certainly to have been inherited from his mother. But he was predominantly *yellow*, and visible yellow is not normally transmitted from a yellow male to his son, so that it is almost certain that he received his yellow also from his mother. But black and yellow segregate in a normal tortoiseshell female. It looks as though our female (4 *d*) were really a yellow, but with some of her yellow-carrying gametes "contaminated" with black[1]. As

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[1] As there is apparently no rule of inheritance in cats without its exception, it is doubtless possible that in this case the yellow father (1) *did* give yellow to his son and that the black *only* came from the mother, in which case she would be a tortoiseshell carrying some unusual modifier of the amount of black, which was also transmitted to her son. It is against the laws of probability, however, that the male (1) should transmit yellow abnormally to a son just on that one particular occasion when he was mated with an unusual female—all his other known offspring were normal *in this respect*. He was, however, the father of 4 *d* as well as of her abnormal son, and this does introduce an element of doubt into the question—he *may* have been responsible for the unusual colouration of 4*d* and in turn for that of her son, in spite of the fact that all his other progeny were normal and that he himself was, in appearance, a perfectly normal yellow: but it seems improbable. By far the most probable explanation of the whole matter seems to us to be that given above, viz. that 4 *d* is abnormal in colouration, *i.e.* is an anomalous yellow and not a tortoiseshell, and that she has handed on her abnormality *in toto* to her son.

such she has, of course, no bearing on Whiting's series, if his animals were all genetically true tortoiseshells. Later she was mated to a different yellow male and produced two yellow males and one yellow female, all without any black-spotting. Unfortunately soon after this she died. Her "anomalous yellow" son had been drowned and preserved in formalin during our absence and his father had strayed and been lost during the same period. That part of our investigation, therefore, came to an untimely end and left us with only a tantalising suggestion.

After the discovery of the black spots on ♀ 4 ♂ we naturally examined all our yellow cats very carefully, but none had the least suggestion of a black spot anywhere. Later one of our yellow kittens became infested with fleas and, when going over it very carefully with a small tooth comb, we found three or four black hairs. No ordinary examination would have revealed this small amount of black and consequently we began to examine every available yellow cat with especial care. We have examined cats from our own stock, from the Cats' Home here in Liverpool, from different parts of England and from the Isle of Man, and the interesting fact has come to light that apparently *all* yellow cats have a few black hairs. Altogether we have examined twenty-six individuals —males, females and neuters (castrated males)—of all ages, and, in all except one newly born kitten, we have been able to find *some* black hairs. In four the black hairs were distinctly ticked as in tabby pattern, and presumably these animals were carrying the factor for agouti (ticking). There is no question of these yellow cats being tortoiseshells near the yellow end of the series; they are ordinary yellows, and in those cases where we have bred from them they have shown no suggestion of segregation between black and yellow. It is doubtful whether a pure yellow cat exists[1]: all are slightly "contaminated" with black. We have also examined forty-three black cats from the same districts as the yellows but have succeeded in finding only one solitary yellow hair; this was on a female.

These findings lead to several points of interest.

In the first place they very strongly suggest that *both* black and yellow are sex-linked. As was pointed out on p. 88, the ordinary facts of inheritance can be explained only by assuming either that *both* black and yellow are sex-linked, or that *one* is sex-linked and the other not, the sex-linked colour being completely dominant in the male, incompletely

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[1] We cannot attach much importance to the one newly born kitten in which we failed to find any black. Often in very young kittens it is extremely difficult to find the black; ten minutes' search with a hand lens may reveal only two or three black hairs.

dominant in the female. The fact that in yellow cats, males and females alike show a few black hairs practically proves that there is no difference in dominance between the sexes, and goes far to establish the sex-linkage of *both* colours. It might be suggested that we are dealing here with a different black from that of an ordinary black cat. Ordinary black in cats is recessive to the ticking which gives tabby. Such recessive black occurs in several other animals, while in some there is a black which is dominant to agouti. In the rabbit both dominant and recessive black are known (Punnett, 1912) and there is good reason to believe that a dominant black exists in cats also. We have not yet had an opportunity of testing the matter, but from Tjebbes' (1924) work on Siamese cats it seems almost certain that such a dominant black does exist. He crossed a Siamese female with a tabby male and got all blacks in the F_1 generation, and these crossed amongst themselves gave some tabbies in the F_2 generation. This F_1 black is clearly not the black of ordinary cats, for that is recessive to tabby. Nothing is known of this dominant black, nor of its place in the general scheme of inheritance of coat-colour in cats[1], but its existence seems fairly certain and it is necessary, therefore, to consider the possibility that this is the black which shows up in yellow cats. If this should prove to be the case, then the scattered black hairs would not necessarily have any bearing on the problem of sex-linkage of ordinary black and yellow. But, as already mentioned, in four yellow cats examined by us, the black hairs were ticked, whereas the dominant black of Tjebbes' cats did not show ticking. It is, therefore, practically certain that these scattered black hairs are not dominant black, and, unless some entirely new facts come to light, it is reasonable to suppose that they are the same recessive black as that of ordinary cats. If this be true, it seems certain that there is no sex-difference in dominance between ordinary black and yellow, and it follows, therefore, that *both colours must be sex-linked*.

This still leaves three alternatives, namely, that black and yellow are a pair of allelomorphs[2], or that black is epistatic to yellow, or that yellow is epistatic to black. On these points, however, we have no real evidence.

There also arises from our results the larger question of the possibility of the splitting of a factor. Although in the present state of our

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[1] Since going to press we have seen a second paper by Tjebbes, 1926, drawing attention to this dominant black in cats and suggesting a possible connection with the problem of the tortoiseshell tom-cat.

[2] This involves the whole question of the validity of the Presence-and-Absence theory. For a discussion of this subject see Bateson (1926).

knowledge we cannot pretend to give an exact scheme of the relationship between black and yellow, it does seem to us that *in any case* our results can best be explained on the supposition of what Bateson (1926) calls the "fractionation" of a factor. Presumably in one of the gametes which went to produce 4 *d*, something happened which caused a small amount of black-spotting to appear in her coat, in spite of the fact that she was evidently what is usually understood by a yellow. It may have been that the greater part of the factor for black was lost, allowing a hypostatic yellow to become visible; or it may have been the partial loss of the factor for yellow, allowing hypostatic black to appear. In either case, partial non-disjunction may have combined with fractionation, causing a part of the factor for one colour to adhere to a chromosome which would normally have determined the other colour. If black and yellow be a pair of allelomorphs such partial non-disjunction is, of course, the only one of the above alternatives which could be applied to our results. Whatever be the exact relationship between black and yellow, however, the fact remains that a quantitative difference in one of them has arisen during the course of our experiments, in 4 *d* and her son, and has evidently arisen also in the past, giving the very small amount of black which is found in all homozygous yellow cats. It seems to us that these quantitative differences strongly suggest the splitting or fractionation of a factor.

Doubtless many workers would prefer to explain our results by linked multiple factors for black, some of which, by crossing-over, might come to lie on the same chromosome as the factor for yellow (or *vice versa*). This line of argument easily leads to absurdity in those cases where the series of known forms passes from one extreme to the other through an unbroken chain of individuals separated from each other only by minute quantitative differences of a single character[1]. Again, it might be suggested that the small amount of black in ordinary yellow cats and the greater amount in 4 *d* and her son are due to restriction factors affecting the black, or extension factors affecting the yellow. This is a possible explanation of the facts under consideration. Unless, however, there is almost perfect linkage between these modifying factors and the colour factors[2], intermediate grades, such as 4 *d* and her son, showing both black and yellow and yet not breeding as true tortoiseshells, should be fairly common, whereas in reality they are extremely rare.

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[1] *E.g.* A complete series exists between black and dominant white in cats. This subject will be discussed in a separate paper.

[2] To suppose almost perfect linkage makes this only a specialised case of multiple factors.

It seems to us more reasonable to accept "yellow," 4 *d*, and black as a series of multiple allelomorphs, which are only different degrees of a single character, and to suppose that *these different degrees have arisen by the splitting of a single factor*. The only difficulty in the way of such an explanation is the deeply rooted but purely hypothetical conception of an indivisible gene.

SUMMARY.

(1) The normal facts of inheritance of black, yellow and tortoiseshell can be explained only by supposing either that both colours are sex-linked, or that *one only* is sex-linked and completely dominant to the other in the male, incompletely dominant in the female.

(2) Black hairs have been found in all yellow cats of either sex examined by us, and this practically disproves any sex-difference in the dominance of black and yellow. There remains, therefore, as the only alternative, the sex-linkage of both colours.

(3) In the course of our investigations a yellow female appeared, showing and transmitting a very small amount of black-spotting which did not segregate from the yellow. We subsequently found that *all* yellow cats have a few black hairs. These facts suggest that in the past and again during our experiments there has been fractionation of a factor.

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CHROMOSOME BEHAVIOUR IN MALE AND FEMALE INDIVIDUALS OF *VALLISNERIA SPIRALIS* AND *NAJAS MARINA*

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(With Two Plates and One Text-figure.)

THE two species of plants mentioned in the title of this paper are both distinguished by remarkable cytological features, connected with the variation in chromosome equipment, as will appear from the following.

Vallisneria spiralis *L.*

I have already shown, in a previous work (1923) that in *Melandrium album, Humulus lupulus* and *H. Japonicus*, a pair of sex chromosomes was to be found. On reduction division in the pollen mother cells, an X-Y pair, with partners of unequal size, was observed. In the case of *Melandrium*, this has since been checked, and the investigation further extended, by several other workers. I had also taken up *Vallisneria spiralis* for purposes of similar research, having studied in particular the chromosome conditions on division of the primary pollen nucleus, which offered clearer views than the reduction division itself. I observed in this connection that the pollen contained sometimes 8 chromosomes, to wit, 2 long, 2 medium and 4 short; sometimes, in addition to these, a peculiar, highly constricted double chromosome, apparently composed of 1 long and 1 short. I therefore stated that it must be regarded as beyond question that this peculiar double chromosome was a sex chromosome, X, found only in half the pollen, and that the sex chromosomes in *Vallisneria* were thus of the *Protenor* type, the male plant having presumably $16 + X$ and the female $16 + X + X$ in diploid cells. In view of the pronounced constriction observed in the supposed sex chromosome, I stated at the same time that it might possibly, in somatic divisions, be separated up entirely into two distinct chromosomes, so that the male plant might be expected to have 18, the female 20 chromosomes.

It was now desirable to test the correctness of this view, and such a test has been made, by C. A. Jørgensen, who has had, *inter alia*, my

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own preparations to work on, and who has communicated to me the results which he describes in the present issue of the *Journal of Genetics*.

As it appears from his researches, with root tips of male and female plants of *Vallisneria spiralis*, he found, contrary to what I had expected, 20 chromosomes in somatic cells in both sexes, and, altogether, entire uniformity between the sets of chromosomes in male and female. This result necessitated further investigation of my own material, and rendered a study of reduction division desirable, for it was beyond all question that pollen with 8 chromosomes did occur. The very fact that I always found 8 or 10 (taking the double chromosome as two) and that it was thus the presence or absence of the double chromosome which determined the difference between the two types of pollen, was, to my mind, a decisive argument in favour of the view that we had here to deal with a special sex chromosome, composed of a larger and a smaller one.

Somatic divisions in Vallisneria. Fixed material of root tips gave results in accordance with those of Jørgensen. Both male and female plants contain 20 chromosomes in somatic cells. Of these I find 6 long, 2 fairly long, 2 rather short and 10 quite short. For the haploid chromosome set in that pollen type, which contained the highest number of chromosomes, I gave (1923) 2 long, 2 medium and 4 short, plus the double chromosome consisting of 1 long and 1 short. In other words, I had formerly found 3 long, 2 medium and 5 short. The two noted as medium in 1923 are however, as it now appears, not alike, one being a good deal shorter than the other. Apart from this, the somatic cell nuclei have proved to contain exactly twice the chromosome set of the pollen. Pl. VII, fig. 1, shows a metaphase in the female plant with 20 chromosomes, while Figs. 2, 3 and 4 show precisely similar nuclear plates from the root tips of the male plants.

The presence of 20 chromosomes of the lengths noted in both sexes shows, in the first place, that the double chromosome which I regarded as a sex chromosome divides into two in the diploid stage, which, as mentioned, I had anticipated would be the case. Further, the presence of identical sets in male and female disproves the correctness of my former view that the double chromosome was a sex chromosome. It remains then to ascertain how pollen with 8 chromosomes can arise, and this we will now proceed to consider.

Nuclear divisions in pollen mother cells of Vallisneria. I had up to now, using Carnoy's method of fixing, obtained but poor views of the reduction division, which I was therefore previously (1923) prevented from studying further. With the aid of Navashin's fixing liquid, which

gives a very slight contraction, it has now proved possible to follow the progress of reduction division more closely, and thus to find the probable explanation of the occurrence of pollen with 8 chromosomes. The preparations were stained for the most part with iodine-gentian-violet.

In the material previously investigated (1923) I found, when there were 10 chromosomes in the pollen, 2 of them invariably united in the oft-mentioned double chromosome. In material subsequently examined, however, I find, in agreement with Jørgensen, that they are often separate. This peculiar variation in the conditions observed is most distinctly paralleled in the case of *Najas marina*, where again 2 chromosomes are sometimes joined together, sometimes found apart, as will be seen when we come to deal with this plant.

Just as the 10 chromosomes in pollen are sometimes all separate, sometimes grouped as 8 + 1 double specimen, so also reduction division shows, in some cases 10 gemini, in others 8 + 1 double one. Pl. VII, figs. 5 and 6, represent heterotypic metaphases with 10 gemini, the first viewed from the pole, the other obliquely from above. In fig. 5, one of the pairs of chromosomes lies horizontally, and is bent to an angle, the others being foreshortened. This horizontal position is presumably due to the fact that the spindle threads are attached close to the middle of the chromosome in question. Pl. VII, fig. 7, shows a heterotypic metaphase with only 9 units, all of which, in polar view, appear foreshortened. A study of the heterotypic division, side-view, also shows that there may often, probably in most cases, be 9 units, one of which however is of peculiar shape, as if double, and lies as a rule horizontally in the nuclear plate. A peculiarity of this double chromosome is the fact that it divides later than the rest, and is just undergoing reduction division when the other 8 have already reached the poles. Pl. VII, fig. 8, shows a heterotypic metaphase in side-view, with a double chromosome lying at right angles to the nuclear spindle. Pl. VII, fig. 9, shows this double chromosome at the stage of incipient fission. It will be noticed that the linin filaments are not attached in the middle of the chromosome, but nearer one end than the other, so that, as the division proceeds, two arms of unequal length appear on the chromosome pair. Pl. VII, fig. 10, shows the same remarkable element together with 8 others, all single. The appearance of the anaphase will be seen from Pl. VII, fig. 11, where 8 chromosomes are shown lying freely at either pole, while the delayed double chromosome pair is still in process of reduction. It is here very clearly apparent that two spindle filaments are attached to this element. The telophase is shown in Pl. VII, fig. 12,

where the double chromosome pair is still situate apart from the 8 others, the latter having long since reached the poles.

The reduction division, at any rate in my material, is thus characterised by the fact that we find in most cases a double chromosome pair with retarded division; and there can be little doubt but that this answers to the double chromosome observed in the pollen itself. My view of the course of reduction division as far as the double chromosome is concerned will be seen from the schematic series in Pl. VII, fig. 13.

When we remember that this remarkable body is very much behindhand in the heterotypic division, it is not surprising that we should often find pollen which has lost the double chromosome altogether, as for instance when the daughter nuclei round off before this element has had time to reach the poles. This, I think, explains why, in my previous investigations, I found sometimes 8, sometimes 10 chromosomes, but not 9 on division of the primary pollen nucleus. This gives rise to the two types of pollen, though it has nothing whatever to do with sex determination.

Altogether, reduction division in *Vallisneria* appears to proceed somewhat uncertainly; and not infrequently, vagabond chromosomes are discovered in an extra-nuclear situation in the cytoplasm. It is extremely probable, then, that pollen with 8 chromosomes is quite incapable of functioning at all.

Najas marina *L.*

The chromosome conditions of this plant have been investigated several times already (Guignard, 1899 *a*, *b*; Müller, 1912; Tschernoyarow, 1914), but nothing has been observed which could suggest the presence of a morphologically demonstrable pair of sex chromosomes, despite the dioecia. We do find, however, cytological conditions resembling those noted in the case of *Vallisneria*, the stock of chromosomes being subject to considerable variation, as will be seen in the following.

Guignard (1899 *a*, *b*) found, on reduction division in pollen mother cells, 6 gemini, but mentions having occasionally met with 7 (1899 *b*). He fixes the diploid number at 12, some being twice as large as the others.

Müller, *l.c.*, whose material was obtained from Moselle finds $2n = 14$, and thinks that Guignard's 12 must be due to working with too low powers of magnification. This explanation seems unlikely, inasmuch as the chromosomes in *Najas* are of such an extraordinary size that they can be studied even when but very slightly magnified. He states that

he has found four different sizes in these 7 pairs of chromosomes, viz. about 14, 8–9, 4 and 1·5μ.

From his description and his Figs. 23 and 24 it appears that these 7 pairs vary according to size and shape as follows: 1 pair very long, V-shaped (14μ); 3 pairs of medium length with hooked points (8–9μ), 2 pairs rather short, slightly curved (4μ), one of which is drawn as a little longer than the other—and finally, 1 pair quite short (1·5μ). The last pair can be attached by a delicate filament to the ends of one of the medium-sized pairs (Müller's Fig. 22), but the writer himself regards this as accidental.

Tschernoyarow (*l.c.*) whose Fig. 1 is here reproduced (Pl. VIII, fig. 14) agrees in the main with Müller, but always sees the smallest pair attached to the end of one of the medium-sized pairs as satellite chromosomes, and gives the following further characterisation:

1 pair very long, V-shaped, with arms of equal length connected by a kind of joint (*aa* in Text-fig. 1, upper row);

3 pairs of medium length, viz.:

1 pair (*b*) to which the smallest pair (the satellites) are attached,
1 pair (*d*) with a slight constriction in the proximal part (seen from the centre of the nuclear plate),
1 pair (*e*) with hooked curvature at the proximal end;

2 pairs of smaller ones, viz.:

1 pair (*f*) with constriction below the middle,
1 pair (*g–h*) a little smaller, with two constrictions, and thus as it were three-jointed;

1 pair (*c*) quite small, being the satellites attached to the pair, *b*, of medium size.

The only demonstrable difference, then, between Müller's and Tschernoyarow's description of the chromosomes in *Najas* is, that the latter constantly, the former only "accidentally," finds the shortest chromosomes attached to a pair of medium size.

Despite the diploid figure 14, Tschernoyarow nevertheless always found but 6 gemini in the heterotypic division, and he concludes from this that the smallest pair of chromosomes really is joined with the pair to which it appears attached as satellites in somatic cells. We have thus here again precisely the same variation as that just noted in the case of *Vallisneria*, where an entirely similar pair of satellite chromosomes, as we have shown, is found, sometimes associated with a larger pair, sometimes isolated in somatic cells. There is further correspondence in the

fact that Guignard occasionally found 7 gemini, albeit 6 was the rule, in the pollen mother cells, and that I have found sometimes 10, sometimes 9 gemini in *Vallisneria*. Both these observations are undoubtedly correlated to the fact that the pair of satellite chromosomes is sometimes isolated, sometimes associated with another pair.

I have, however, in my own researches with *Najas marina*, found chromosome features which do not by any means agree with previous observations for this plant, and which show, firstly that the chromosome equipment may be different from that already mentioned, and further, that my material has sometimes 12, sometimes 14 in somatic cells, according to whether two pairs of chromosomes are united or not. Despite a certain agreement in principle, viz. as regards the variation in the chromosome number, there are nevertheless conspicuous differences as regards the size and structure of the individual chromosomes themselves.

The plants on which my investigations were based were collected at Köge, on the island of Sealand, one of the two localities in Denmark where the species is found. The material examined consisted partly of root tips, of male and female plants, and partly of pollen mother cells in reduction division.

Somatic divisions in Najas. The chromosomes in my material, though resembling, to a superficial view, those previously found, exhibit nevertheless conspicuous and essential differences (Pl. VIII, figs. 15–23). I find, in both male and female plants:

5 pairs (*a–f*, Text-fig. 1, lower row) of long chromosomes, all with more or less pronounced constriction and curvature at the proximal end (towards the middle of the nuclear plate). Some of these have also a constriction above that already noted. The length is not entirely uniform throughout, but accurate subdivision according to length is out of the question.

1 pair (*g*) of short chromosomes, the size of these amounting to only about one-fourth that of the long ones.

1 pair (*h*) very short, even smaller than the last mentioned, with which they are often connected, the whole then resembling one constricted chromosome.

It is clear from the foregoing that the diploid nuclear plates will exhibit 12 or 14 chromosomes, according as the two smallest pairs of chromosomes are connected or not; and these different chromosome numbers appear very distinctly even in one and the same root tip, and

in both male and female plants. Pl. VIII, figs. 15–18, show a series, from altogether united to altogether isolated minor chromosomes in the male plant. Pl. VIII, figs. 19–23, gives a corresponding series for the female.

The V-shaped pair of chromosomes described by previous writers is not found in my material. But it seems evident to me, and plainly visible in Tschernoyarow's figure (here reproduced as Pl. VIII, fig. 14) that the chromosomes which constitute this pair are themselves composed of two long chromosomes, which would sufficiently explain the V-shape. And I would call to mind, in this connection, the difference found, for instance, between the X-chromosomes of *Drosophila melanogaster* and *D. obscura*. But in other respects also it is obvious that my material differs from that previously described, though I find it hard to
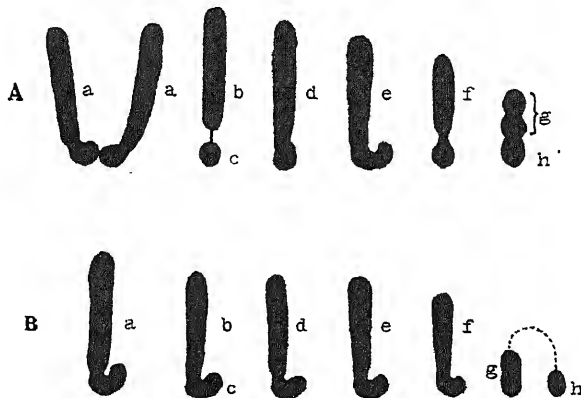
Fig. 1. Showing the probable homology between the chromosome equipment of the two types of *Najas marina* L. *A*. The type of Guignard, Müller and Tschernoyarow. *B*. The new type.

see how the one type can be derived from the other. What seems to me most likely is, that: (1) Müller's and Tschernoyarow's type, which we will call *A*, differs from mine, *B*, in that one of *B*'s long chromosome pairs appears in duplicate in *A*, thus producing the peculiar V-shaped chromosome (see Text-fig. 1); (2) the satellites in the *A* type are, in *B*, always completely united with the pair of chromosomes to which the satellites belong, so that only a constriction remains; (3) the smallest (save for the satellites) constricted pair of chromosomes in *A* is in *B* sometimes divided into two, sometimes a unit.

Considering the size and shape of the chromosomes in the two types, this seems to me the most reasonable explanation, though it is here put forward only as a working hypothesis for future research. Judging from

the cytological picture presented, the type I have investigated would really be regarded as specifically distinct from the others.

Divisions in the pollen mother cells of Najas marina. Like Tschernoyarow, I have always found but 6 gemini in the heterotypic division in pollen mother cells, as shown in Pl. VIII, figs. 24 and 25, representing early and late anaphase respectively. The smallest chromosome is as a rule easily distinguishable from the rest, but not always. The spindle filaments are attached subterminally, which is particularly apparent in the telophase (Pl. VIII, fig. 26) where the ends of the chromosomes nearest the poles are bent over, approximately as in somatic nuclear plates.

The homotypic division is rather difficult to view as a whole, owing to the fact that the chromosomes here lie close together, and are unusually long.

SUMMARY.

The fact that *Vallisneria spiralis* pollen, as I have previously pointed out (*l.c.*) contains sometimes 8, sometimes 10 chromosomes (*i.e.* 8 + 1 double) is not, as I had supposed, a feature connected with the sex determination; both Jørgensen (*l.c.*) and I have found the same chromosome equipment (20 chromosomes) in the somatic nuclear plates of male and female plants. The appearance of 8 or 10 chromosomes is due to the fact that the double chromosome, at any rate in my material, is lagging behind the others at the reduction division and may quite frequently altogether fail to enter into the cell nuclei of the pollen, which will then have but 8 chromosomes. Such pollen must be regarded as incapable of functioning at all.

The double chromosome in question appears on reduction division in pollen mother cells sometimes as a single element, sometimes as two separate ones, so that sometimes 9, sometimes 10 gemini can be observed.

In *Najas marina* we similarly find, in somatic cells, sometimes 12, sometimes 14 chromosomes, as here also one pair of chromosomes may occasionally be united with another, thus reducing the number to 12. On reduction division, we find here as a rule only 6 gemini; Guignard, however (*l.c.*), has occasionally found 7. No difference in chromosome equipment of male and female plants could be found.

The *Najas marina* type investigated by me differs in regard to individual shape and size of the chromosomes from those previously investigated, and this to a conspicuous degree.
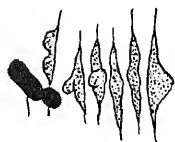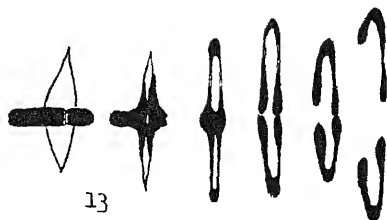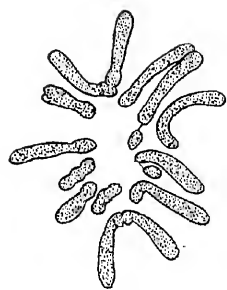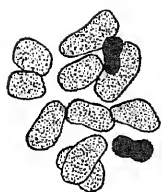
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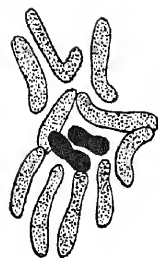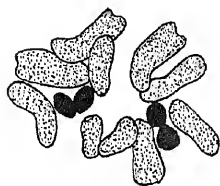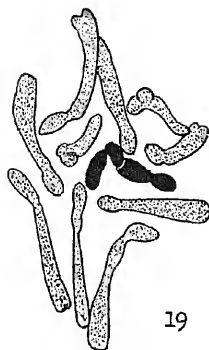
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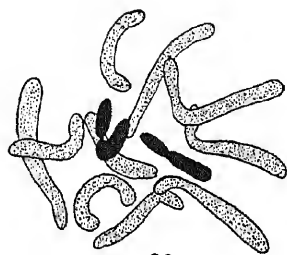
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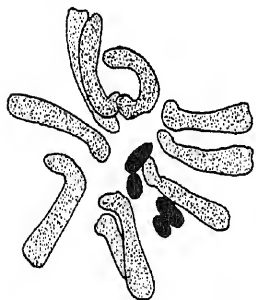
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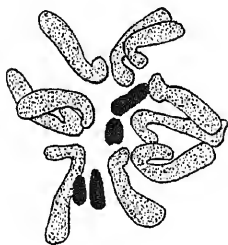
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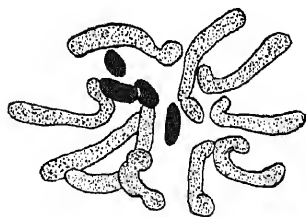
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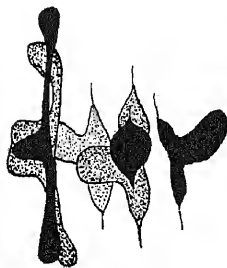
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EXPLANATION OF PLATES VII AND VIII.

All figures are drawn with aid of camera lucida. Magnification about 2000.

PLATE VII.

Vallisneria spiralis L.

Figs. 1. Somatic metaphase from root tip of a female plant.

Figs. 2, 3, 4. The same from male plants.

Figs. 5–12. Heterotypic plates from pollen mother cells.

Fig. 5. Metaphase with 10 gemini, polar view.

Fig. 6. Metaphase with 10 gemini, obliquely from above.

Fig. 7. Metaphase with 9 gemini, polar view.

Fig. 8. Metaphase. The double chromosome (black) lying at right angles to the nuclear spindle. Not all chromosomes are figured. Side-view.

Figs. 9, 10. Metaphases with 9 chromosome pairs, one of which (at left), is double.

Figs. 11, 12. Late anaphase and telophase respectively, with 9 chromosome pairs, one of which (black) is double and lagging behind.

Fig. 13. Schematic series of the reduction division of the double chromosome.

PLATE VIII.

Najas marina L.

Fig. 14. Copy of Tschernoyarow's Fig. 1, of a somatic plate from a female plant, polar view.

Figs. 15–23. Somatic metaphases from root tips, all in polar view. Figs. 15–18 from male plants; figs. 19–23 from female plants. The two smallest chromosomes (given in black) are more or less united or entirely separate. Hence the diploid chromosome number is varying from 12 to 14.

Fig. 24. Heterotypic metaphase from pollen mother cell, with 6 pairs of chromosomes. The small one in the middle (black) is the double chromosome.

Fig. 25. Heterotypic anaphase from a pollen mother cell, side-view. The small (double) chromosome visible at both poles.

Fig. 26. Homotypic ana-telophase from pollen mother cell, side-view. The short (double) chromosome (black) visible at both poles. One of the long chromosomes at the upper pole (black) has been cut through.

FERTILITY IN THE GENUS *BRASSICA*.

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(With One Plate and Seven Text-figures.)

CONTENTS.

INTRODUCTION.

THE cross- and self-compatibility of most natural groups of plants have always excited considerable interest, principally, however, because of their bearing on phylogenetic relationships. The present study deals with the question of fertility in the genus *Brassica* from the point of view of economic plant breeding and seed production.

The genus is of considerable importance commercially and possesses great potentialities for the practical plant breeder. In planning any extended scheme of constructive plant breeding it is helpful to know what is crossable and what is not. In commercial seed production, especially in a country such as this, where the industry is extremely localised, it is essential to know the degree of crossing which may take place in the field, because this ultimately controls the spacial isolation of crops.

Within the genus *Brassica* (including *Sinapis*) there is a large number of cultivated species, each embracing many highly specialised varieties. These varieties have to be grown, flowered and harvested in a high state of genetic purity for certain definite significant characters. To the grower who would produce "pure" seed of as many *Brassica* types as possible on a limited area, and to the plant breeder working on the genus, the questions of "crossability" and "self-fertility" are therefore of prime importance.

The amount of crossing which takes place in the field in a given time equals PC, where P is the foreign pollen incident on the aggregate stigmatic surface of the crop in question, and C a constant applicable to any two crops involved.

It is difficult to determine a value for P, though not impossible. It depends on a large number of variables, such as the size of the crops involved, the incidence of a significant insect population, the general direction of the wind in relation to the crops at the time of flowering, etc. Clearly, C can never be greater than unity, and, when it reaches this, the amount of rogues produced will be subject only to the factor P. In cases when the value of C has been found to be at the maximum the most important step is to decide the minimal spacial relationship which will render P equal to zero, and so allow genetically pure seed to be produced. The step of greatest practical importance, however, is to arrive at a value for C, because, obviously, where this is definitely zero, practical difficulties regarding spacial isolation of the crops vanish.

It may be noted here that the importance of self-sterility lies in the fact that pollen from a source external to the plant must be brought in. Thus, in the event of out-varietal pollen being incident on any plant, the chances of cross-fertilisation are controlled by the degree of compatibility of the cross. In wheat, which, normally, in this country, only very rarely crosses, Watkins[30], found that certain more or less self-sterile hybrids crossed freely in the field. In self-compatible plants there may be a bias against crossing due simply to the home pollen being completely effective. It is seen, then, that C, for any one variety in relation to another, is not simply the degree of cross-compatibility which may exist between the two, but is the resultant of their cross-compatibility with the self-compatibility of the one in question.

The most obvious method of estimating crossing would appear to be to grow plants of the various forms in various spacial relationships and get a result by counting the aberrant types in the progeny. This method suffers from a number of disadvantages. In the first place, all the

variables included under P are operative. Secondly, results from the
precise study of the heredity of *Brassica* have only lately begun to appear,
and, as much of the available commercial material seems to be somewhat
unstable, and probably not of high genetic purity apart from the signifi-
cant commercial characters of the variety, the variations observed might
give quite misleading results. In the producing areas under the local
conditions to which the results must apply the promiscuous planting of
Brassicas is not desirable. The methods reported here are much more
laborious, but it is hoped will eventually give precise results, and when
coupled with studies of natural pollination factors will yield accurate
data for field isolation.

SELFING.

For the selfing and all other work requiring isolation, "Glassene"
paper blossom bags made under the personal supervision of the writer
were employed. They were thus always of ample size and suitable shape.
It is noteworthy that the self-sterile types which did not set a single
seed when selfed under one of these bags set ample seed under an
exactly similar bag when impregnated with pollen from a compatible
fellow, the two lots of flowers blooming simultaneously.

A number of questions all more or less related to each other and
to the evaluation of the constant C were involved in the selfing work.
Firstly, it was required to know how far the plant's own pollen was
effective when a sufficiency was available to the stigma. Secondly, there
is a current belief that all the pollen available on the plant at a given time
is not equally effective for fertilisation. That is to say, pollen brought
from one flower to another on the same plant is supposed to be more
effective than pollen simply transferred from the flower's stamens to
its own stigma. Darwin (8), however, showed that in *Ipomoea purpurea*,
in one sense at least, the converse is true, strict self-fertilisation (flower
to itself) resulting in more vigorous progeny than by the mating of flowers
on the same plant. Darwin did not note the fertilisation itself: he noted
the vigour of the resulting progeny, though, as will be indicated later,
there seems to be some relationship between vigour of fertility and the
vigour of the resulting progeny. Price (24), when discussing a cabbage
hybrid, says, "Attempts to self-pollinise individual flowers resulted in
failure. It was also found practically impossible to secure seed by crossing
different flowers on the same plant."

A comparison of the two degrees of pollination from the point of view
of seed production was embarked upon. This is not so much a study of

pollination as a study of pollen conditions and relationship to the stigma. Through all these selfing experiments "Method 1" refers to the hand selfing of the individual flowers of a whole spike under a bag, no attempt being made to protect the flowers one from the other, though, of course, all natural aids to pollination were ruled out. "Method 2" refers to the hand transference of pollen from one flower to the stigma of another on the same spike under a bag[1], no effort being made to prevent the flowers selfing provided they could. The hand transference of pollen in practice takes a considerable amount of time, and in routine work introduces the possibility of error due to contamination. To find out if this could be eliminated a third method was introduced. "Method 3" refers to a whole spike being simply enclosed in a bag, securely fastened, and never removed until all the flowers had fallen. Every plant was noted by all three methods, the three tests on each plant running concurrently.

Method 1 ensured to each flower on a protected spike a supply of its own pollen. Method 2 ensured to every flower on a protected spike a supply of pollen from at least one other flower on the same spike, while Method 3 left the flowers on a bagged spike without aid from any pollinating agent, natural or artificial, apart from the shaking of the plant as a whole in the wind.

In Table I are given the results of selfing the parental types. The number of flowers protected is the denominator, and the number of pods produced the numerator of a "fraction," while the number of these pods containing seed and the average amount of seed per pod are given in separate columns.

TABLE I.

Results of selfing parental types.

Kind and year	Method*	Pods set / flowers bagged	Pods with seed	No. of seeds per pod	Remarks
Cabbage 1924.	1	$\frac{70}{73}$	No seeds	—	Another plant of
var. Early	2	$\frac{68}{70}$	,,		same var. gave a
Enfield Market	3	$\frac{53}{75}$	,,		similar result
Cabbage 1925. Scotch York	2	$\frac{176}{176}$	No seeds		
Cabbage 1925. Drumhead	2	$\frac{160}{165}$	12	1–2	The seeds were all at the distal end of the pods

* See text.

[1] Pollen was transferred with sterile forceps, a whole burst stamen being grasped and used to touch the desired stigma. Forceps were wiped when changing from method to method and sterilised with spirit when passing from plant to plant.

TABLE I (*continued*).

Kind and year	Method*	Pods set flowers bagged	Pods with seed	No. of seeds per pod	Remarks
Cabbage 1926. Daniels' Defiance	2	See special note			
Borecole 1924	1	$\frac{64}{74}$	No seeds		
	2	$\frac{66}{64}$	,,		
	3	$\frac{32}{82}$	,,		
Savoy 1924	1	$\frac{45}{47}$	No seeds		
	2	$\frac{82}{70}$	,,		
	3	$\frac{158}{166}$	,,		
Savoy 1925	1	$\frac{30}{38}$	No seeds	—	Clone from the 1924 plant
	2	$\frac{16}{33}$	,,		
	3	$\frac{23}{54}$	,,		
Brussels sprout 1924. var. Solidity	1	$\frac{30}{33}$	No seeds		
	2	$\frac{34}{40}$	,,		
	3	$\frac{149}{164}$	,,		
Kohl rabi 1924. Green-top	1	$\frac{62}{74}$	All	22	Under Method 3 the fertile pods were situate on the lower portion of the spike
	2	$\frac{80}{88}$	,,		
	3	$\frac{85}{87}$	22	22	
Kohl rabi 1925	1	$\frac{88}{103}$	20	22	Clones from the 1924 plant, *i.e.* 3rd year
	2	$\frac{111}{117}$	30	22	
	3	$\frac{152}{152}$	10	6–12	
Broccoli 1924. White heading	1	Every pod set	No seed	—	Accurate counts not made
	2	,,	,,		
	3	,,	,,		
Broccoli 1925. White sprouting	1	$\frac{12}{114}$	32	5	An unprotected spike set 35 pods from 98 flowers
	2	$\frac{47}{107}$	36	5	
	3	$\frac{20}{85}$	22	5	
Broccoli 1924. Purple sprouting	1	$\frac{30}{84}$	30	22	—
	2	$\frac{45}{155}$	45	22	
	3	$\frac{80}{140}$	80	22	
Swede 1924. "Mervue"	1	$\frac{52}{60}$	52	18	Plant grown as annual
	2	$\frac{41}{49}$	41	18	
	3	$\frac{58}{92}$	58	Variable	
Turnip 1924. Greystone	1	$\frac{25}{65}$	24	16	Plant grown as annual
	2	$\frac{35}{50}$	28	16	
	3	$\frac{0}{123}$	—	—	—
Turnip 1925	1	$\frac{36}{48}$	33	17	Plants grown as annuals from the selfed seed saved in 1924
	2	$\frac{9}{18}$	4	17	
	3	$\frac{18}{24}$	2	3	
Rape 1925	1	$\frac{36}{66}$	33	7–8	Plants grown as biennials
	2	$\frac{24}{46}$	19	7–8	
	3	$\frac{31}{60}$	20	5–6	
Mustard. Brown and white	—	Both the mustards proved to be fully fertile by all three methods			

* See text.

All the "cole" types (forms of *Brassica oleracea*) were grown in the familiar way—as biennials; the turnip, swede and mustards as annuals. An exception to this rule was a certain number of cabbages which flowered in 1926 and are, later, specially mentioned. On systematic grounds at least, there may be taken to be three broad groups within the genus.

Firstly, there is the cole group which includes the cabbages, savoys, brussels sprouts, broccolis, kohl rabi, etc.; secondly, the turnip, swede, rape group; and thirdly, the mustards.

From the point of view of compatibility simply, the cole group would seem to comprise two sections, the first of these, including cabbage, savoy, borecole, brussels sprouts, and white heading broccoli, being totally self-sterile. The second section, containing kohl rabi and the sprouting broccolis, is more or less self-fertile. These data apply to plants grown as biennials, the case of cabbage plants grown as annuals being dealt with later.

A distinction drawn by Kraus and Kraybill [21], and East and Park [11], must be referred to here; that is, between fruitfulness and fertility. Plants producing fruit but no viable seeds are fruitful but sterile; only when seeds are produced are the plants regarded as fertile. Many of the self-sterile plants examined in these studies were highly fruitful, the fruits being here referred to as "false pods." Those pods are quite as large as the normal fully fertile pod, only that they are not plumped out with seed. Emasculated protected flowers produce only very small shrunken pods or none. Focke [13], and Herbert [16], both stress the multiple and distinct functions of pollen, fertilisation of the ovum and stimulation of the fruit.

The members of the second section of the cole group comprising kohl rabi and the sprouting broccolis are self-fertile, though their fertility may be very low. That heading broccoli is sterile while sprouting broccoli is fertile is worthy of note. In the case of kohl rabi, "crossing" two flowers on the one spike would appear to have been rather more effective than simply selfing the individual flowers. This is probably due to differences in the relative maturity of pollen and stigmas; many varieties in the general cole group are known to be protogynous. In the case of the broccolis no definite difference is apparent between the different methods, the highest number of seeds being produced by the untouched bagged spike of purple sprouting broccoli, while the lowest figure is shown by the spike of white sprouting broccoli left unprotected and open to the action of all the natural pollinating agents! This plant

was fairly well isolated from the other *Brassica* plants by intervening rows of sweet peas. The case of the cabbage investigated in 1926 requires somewhat extended description. The plants used were members of an ordinary crop grown for seed—var. "Daniels' Defiance." Sown in April, 1925, and planted out during the following July, flowering commenced in May of 1926, *i.e.* some thirteen months after sowing. The whole area was planted from the same stock seed and all the plants were treated alike. In the field two groups of plants were used. The first group, made up of eight plants, was situated on well-drained soil at the crest of a small knoll. The second group, of five plants, was on the lower portion of the slope. Another plant with white flowers growing in close proximity to the second group was, like all the plants in both groups, selfed by Method 2. The season generally was rather wet, and a common complaint amongst growers was, that seed crops "kept growing" and seemed loth to "pod up." It is probable that Group 2 received rather more moisture than Group 1. It was noticed that a larger proportion of plants round Group 2 failed to shoot than was observed round Group 1. As a general rule a variable number of plants in all annual grown crops of cabbage fail to shoot. This in itself might be regarded as a form of sterility, or, at least, retardation of the reproductive phase. After selfing (Method 2) the following results were obtained:

Plant no.	Pods set flowers bagged	Pods with seed	No. of seeds per pod	
Group 1. Dry area				
1	$\frac{29}{31}$	10	1–6	
2	$\frac{27}{30}$	22	2–22	
3	$\frac{29}{30}$	20	2–22	
4	$\frac{30}{35}$	11	2–4	
5	$\frac{30}{40}$	12	1–22	
6	$\frac{14}{24}$	4	1–3	
7	$\frac{17}{19}$	15	2–22	
8	$\frac{25}{30}$	5	1–2	
Group 2. Wet area				
9	$\frac{17}{26}$	4	1–4	
10	$\frac{24}{29}$	1	5	
11	$\frac{26}{40}$	1	4	
12	$\frac{24}{35}$	3	1–2	
13	$\frac{37}{30}$	1	4	
14	$\frac{66}{73}$	6	1–2	White flowered "rogue"

Consideration of the results shows that as regards fruitfulness both groups are practically equal, but as regards fertility Group 2 (wetter area) is very much lower than Group 1 (drier area). This is in accordance

with Kraus and Kraybill who found that abundant moisture induced sterility. Further, the results support the general practice of seed growers who, as a much applied rule, grow all plants for seed production under rather reduced cultural conditions. Thus, crops when grown in pots are invariably somewhat pot-bound and kept rather drier than the ordinary gardener would like[1]. Also, indoor fruit growers, as, for example, tomato growers, in many cases tend to keep the plants reduced until fruit sets (*i.e.* until after fertilisation), after which they feed the plants liberally in order to produce large juicy fruits. Again, the general practice of growing seed crops of many of the biennial vegetables (mangold, swede, turnip, cabbage, etc.) as annuals would seem to have more than a purely economic aspect[2], in that this too is only another method of reducing the vigour of the "vegetative phase" with, probably, increased fertility. It is to be noticed that functioning gametes per plant is not the interest of the seedsman so much as functioning gametes per unit of area, and therefore though the "annual" plants may be smaller, their increased fertility and greater number per unit of area compensates for their reduced size. Annual habit may involve reduction of the flower number per plant, a point which requires further research. The important aspect from the point of view of these studies, however, is that the value of the constant C is considerably altered by comparatively simple variation of the environmental conditions such as are commonly adopted in practice.

Turning now to the results given by the turnip, swede, rape group, a rather different state of affairs is found. Swede is practically completely self-fertile when a sufficiency of pollen is definitely applied to the stigma. Turnip proved to be rather less fruitful and therefore rather less fertile than swede[3]. Rape behaved in much the same way as turnip. The question of the low results from Method 3 in this group being evidently one of pollination, will be discussed separately. The mustards on all occasions by all methods proved to be fully self-fertile.

[1] Stout (26) has stated that with *Brassica pekinensis* self-compatibility of a plant as a whole or a family of plants may be decidedly changed by a cultural treatment which reduced vegetative vigour.

[2] By growing the main bulk of these biennials as annuals a year is saved; thus land and capital are earlier set free. Stock seed, *i.e.* seed used to produce the commercial crop, is usually borne on specially selected parents fully developed in the usual biennial manner.

[3] Drummond (10), referring to plants at Corstorphine (near Edinburgh), in 1923, says, "All the swedes produced plenty of selfed seed, the turnips on the other hand proved to be comparatively self-sterile."

CROSS-COMPATIBILITY.

The question of the cross-compatibility of the *Brassicae* has received much attention in the past. In the earlier experiments the forms were simply planted together and natural pollinating agents allowed to operate, data regarding the crossability of the types being deduced from the character of the resulting progeny. Little or nothing was known, however, regarding the genetics of the parents. The value of this type of evidence is doubtful, but may be used when the facts are positive.

Concerning first the cole group. Darwin[8], planted a white kohl rabi, a purple kohl rabi, a Portsmouth broccoli, a brussels sprout and a sugar-loaf cabbage near together and left them uncovered. Seeds from each kind were sown in five separate beds. The majority of the seedlings in all the beds were mongrelised in the most complicated manner, some taking after one variety and some after another. The effects of the kohl rabi were particularly plain in the enlarged stems of many of the seedlings.

The same authority planted together two varieties of cabbage with purple-green and white-green laciniated leaves. "Of the 325 seedlings raised from the purple-green variety, 165 had white-green and 160 purple-green leaves. Of the 466 seedlings raised from the white-green variety, 220 had purple-green and 246 white-green leaves." The former of these two cases cited is not altogether precise, but the second seems positive. If the two parent plants were totally self-sterile, as might reasonably be expected, and one heterozygous for purple colour, then the half and half numbers in each case are explained[1].

Focke[13] quotes various authors who indicate some considerable degree of crossing between the members of the genus. Price[24], under strictly controlled conditions, made reciprocal crosses involving heading and non-heading cabbage, savoy, cauliflower, brussels sprout and kohl rabi. No difficulty seems to have been met with in making the crosses. Kristofferson[22] also made many crosses involving the *Brassica oleracea* (cole) group. Sutton[28] arranged the *Brassicae* in three groups according to their fertility when crossed. His third group comprising all the coles, Sutton concluded, was completely cross-fertile. Herbert[16] crossed turnip with swede, using the flower colour of the progeny as an indication of what had happened. An anonymous writer in the *Gardener's Chronicle* for 1885, p. 730, reports on a case where contiguous beds of rape and

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[1] Kristofferson[22], and Pease[23], have shown that purple colour in the leaf is based on a single Mendelian factor pair and its presence is dominant.

turnip flowered at the same time. The seed produced by the turnip, when sown, developed into a lot of mongrels with forked roots, double crowns and some rape-like plants. Ashby(1) reports a case of crossing between crops of swede and rape under field conditions, the damage to the swede stock being assessed at £400. Sutton(28) crossed swede with turnip reciprocally under controlled conditions and obtained seed. The two aspects of the cross were, however, not the same. When the swede was fertilised by the turnip, that is to say, when the swede was used as the seed-bearing parent, abundant, plump, black-coated seed typical of the swede was obtained. When, however, the turnip was fertilised by the swede, *i.e.* when the turnip was made the seed parent, the seed produced was small, shrivelled, and difficult to germinate.

Sutton surmised that many other crosses involving swede, turnip and rape would behave in a similar way. Kajanus(17) crossed swede and turnip reciprocally.

No case of crossing between the two mustards commonly grown in this country, or between them and the other *Brassicae*, occurs in the literature.

Focke(13) quotes Sageret, who reported a successful cross between turnip ♀ and cabbage ♂ (*Brassica napus* L. ♀ × *B. oleracea* L. ♂), and also between rape ♀ and cabbage ♂ (*B. rapa* L. ♀ × *B. oleracea* L. ♂). Focke does not seem to be convinced. Baur(3) states that in this way crossing radish with cabbage is easy, but that the crossing of cabbage with turnip and also the reciprocal has never succeeded in spite of many experiments. Karpetchenko (20) found the mating *Raphanus sativus* L. ♂ × *Brassica olearacea* L. ♀ to be sterile, while the reciprocal yielded a variable F_1 which was always sterile. The present writer made all the crosses shown in Table II with the attached results. The examples given from the cole group support completely the general conclusions of Sutton (28) and others, that the members of this whole group are inter-fertile. Some indication, however, is made here that there is a variation in the degree of fertility between them.

This variability all centres round what has always been looked upon as the stem line of the group—the cabbage itself. Thus the savoy is not so fertile with cabbage pollen as with foreign savoy pollen, while the cabbage reacts to savoy equally well as to pollen from another cabbage. There seems to be some indication of a difference in the reciprocal crosses. (Stout(25) has noted that reciprocal crosses between pairs of plants may give opposite results.) While purple sprouting broccoli is totally fertile with white heading broccoli either way, it is

TABLE II.

Results of crossing parental types.

Pollen parent		Seed parent	Result	Remarks
Savoy	×	Cabbage	Practically complete fertility	Cabbage is as fertile with savoy pollen as strange cabbage pollen
Cabbage	×	Savoy	,,	
Purple sprouting broccoli	×	Cabbage	50 % of seeds in pod. All pods set	—
Cabbage	×	Purple sprouting broccoli	Rather less fertile	—
Brussels sprout	×	Cabbage	Set about 70 % of seed in pods	—
Cabbage	×	Brussels sprout	Practically complete fertility	—
Borecole	×	Cabbage	Complete fertility	—
Cabbage	×	Borecole	,,	—
Brussels sprout	×	Kohl rabi	,,	—
Kohl rabi	×	Brussels sprout	,,	—
Purple sprouting broccoli	×	Kohl rabi	,,	—
Kohl rabi	×	Purple sprouting broccoli	,,	—
White heading broccoli	×	Purple sprouting broccoli	,,	—
Purple sprouting broccoli	×	White heading broccoli	,,	—
Swede	×	Turnip	,,	Seeds shrivelled
Turnip	×	Swede	,,	Seeds plump
Rape	×	Swede or turnip	,,	—
Swede or turnip	×	Rape	,,	—
Brown mustard	×	White mustard	No seeds, small pods	The flowers fell early
White mustard	×	Brown mustard	Neither seeds nor pods	,,
Brown mustard	×	Borecole	No seeds, small pods	,,
Borecole	×	Brown mustard	Neither seeds nor pods	No effect
White mustard	×	Turnip	No seeds, small pods	Pods ripened quickly
Turnip	×	White mustard	Neither seeds nor pods	—
White mustard	×	Swede	No seeds, small pods	Pods ripened quickly
Swede	×	White mustard	Neither seeds nor pods	No effect
White mustard	×	Charlock	Neither seeds nor pods	—
Charlock	×	White mustard	No seeds, small pods	—
Brown or white mustard	×	Swede × turnip F_1	No effect	—

only 50 per cent. fertile in either direction when crossed with cabbage. Again, brussels sprout is completely fertile with kohl rabi, but only 75 per cent. fertile with cabbage.

Turning to the turnip, swede, rape group much the same state of affairs occurs: differential fertility between different crosses involving the same types is found, and the turnip-swede case cited by Sutton is noted. The two mustards (brown and white) did not cross with each other nor with any other *Brassica* included in these studies. The physiological result of pollination with mustard pollen on various Brassicas such as turnip, will be discussed later.

THE WORK WITH PARENTAL TYPES.

This part of the work, principally concerned with the chances of "rogue" production apart from questions of pollination, has shown that in the cole group of the genus there is a section comprising forms which, if not always, are nearly always, completely self-sterile when grown in the ordinary way as biennials, even though the gametes be perfectly viable in compatible relationships. In these types, then, every seed must be of "crossed" origin. Thus, the character of the progeny depends in large part on the character of a pollen supply produced *external* to the plant, the chance or ability to self-pollenise which may exist being of no account. A plant completely surrounded by plants genetically similar to itself and therefore unlikely to receive out-varietal but compatible pollen will reproduce its like, but the slightest influx of dissimilar pollen must ultimately show in the progeny. The proportion and nature of the rogues will of course depend entirely on the amount and nature of the foreign pollen incident on the stigma. The example of the leaf character in two cabbages already cited from Darwin is peculiarly apt in this connection. In the other section of the cole group which is more or less self-fertile, though still largely cross-compatible, there are greatly reduced chances of natural crossing, because here the fertilisation is not wholly dependent on an externally produced pollen supply. The relative "fertilising value" of various types of compatible pollen as compared with "home" pollen will require some attention, because, as Herbert(16) observed with *Rhododendron* and *Azalea*, two compatible pollens may not act with equal rapidity on the same stigma.

Looking, then, at the genus as a whole it may be said that the constant C is highest in relationships involving the completely self-sterile, highly cross-compatible section of the cole group, and zero in the highly self-fertile, completely cross-incompatible mustards, those

forms showing partial self- or cross-compatibility taking an intermediate position.

RESULTS OF THE VARIOUS CROSSES.

The next step taken was to test the compatibility of the hybrids resulting from the crossing experiments just reported; also to try crosses back to parental types, and out to other types in various relationships. The simplest procedure will be to discuss briefly each hybrid in turn, referring to its self- and cross-compatibility, etc. The estimates of cross-fertility are necessarily arbitrary, but probably have a useful degree of accuracy. All the lots of hybrid seed were sown in early August of the year of harvest, and the plants, as soon as fit, planted out in the field. During the winter 1924/25 there was a very small death rate, and some of the lines flowered as "annuals" in the spring of 1925. All the plants were left in the ground, and during the winter of 1925/26 there was a rather heavy death rate. All the lines, however, which did not flower in 1925 survived and flowered in 1926 with only one exception. Hence there is some record either as "annual," "biennial," or both for every hybrid noted in Table II, with one exception. Selfing was done in the manner already described for the parental types.

Cabbage × savoy. Both aspects of this cross were alike in appearance, being "weakly" savoy as reported by Price [24]. The F_1 which had cabbage as the seed parent flowered in its first year (annual) and was selfed with the following results:

Method	Pods set. Flowers protected	Pods containing seed	No. of seeds in the fertile pods
1	$\frac{52}{56}$	4	2–8
2	$\frac{44}{55}$	2	2
3	$\frac{10}{55}$	0	—

The increased pod production following on deliberate pollination similar to that found in the parental types is again to be noted along with the practical sterility of the plant. The reverse of this cross did not flower as an annual and both lines died out in the second winter.

Cabbage × purple sprouting broccoli. Both aspects of this cross were similar in appearance and had broccoli leafage. There was no sign of a "curd," a normal cole inflorescence being produced in the usual way. In the F_1, which did not flower until its second year, the leaves during the first summer turned over and inwards as if to afford protection to a "curd." This gave a semblance of "heading." The sepals of the flower were deep purple like those of the broccoli parent. The F_1 plants which

had the broccoli as seed parent flowered in the first year, persisted, and flowered again in the second summer. Those with cabbage as the seed parent flowered only in the second year. The results of selfing are as follows:

Method	Pods set. Flowers protected	Pods containing seed	No. of seeds in the fertile pods
Cabbage ♂ × purple sprouting broccoli ♀, 1st summer (annual)			
1	$\frac{50}{116}$	No seed	
2	$\frac{46}{106}$	,,	
3	$\frac{51}{109}$	,,	
Same plant, 2nd summer (biennial)			
1	$\frac{39}{90}$	5	1–2
2	$\frac{60}{70}$	4	1–2
3	$\frac{32}{74}$	9	0
Purple sprouting broccoli ♂ × cabbage ♀, 2nd summer (biennial)			
1	$\frac{28}{58}$	No seed	
2	$\frac{53}{53}$	,,	
3	$\frac{35}{40}$	,,	

The interesting feature is that this is the F_1 of the mating of a completely self-sterile form with the form which gave the highest figure for self-fertility in the parental tests. Crossed with broad-leaved rape the hybrid ex cabbage ♀ produced aborted shrivelled seed when used as the seed parent, but was sterile when used as the pollen parent.

Brussels sprout × *cabbage*. Reciprocals were alike in appearance, and when mature produced an open loose head and open loose sprouts. The leafage of the plant was carried up some way on a bare "leggy" stem. Neither F_1 bloomed in the first year, and neither produced a single seed when selfed by the three methods in the second year, though every flower protected produced a pod by Method 1, not so many by Method 2, and still less by Method 3.

Crossed with rape this hybrid was quite ineffective as the pollen parent, but as a seed parent it produced weak, shrivelled, non-viable seed.

Cabbage × *borecole*. This hybrid was weakly "heading," and had typical kale leaves. It was the most winter-hardy of the hybrids. Both aspects of the cross flowered in their second summer only, and gave results very similar to those described for the last cross. They developed 11 seeds from 315 flowers protected by all methods. Crossed with rape it produced aborted, shrivelled seed when used as the seed parent, but proved quite sterile when used as the pollen parent. Crossed with the four hybrids involving matings of turnip with rape and swede with

rape it was sterile every way. The pods produced by the pollen of these four hybrids were very similar in size and appearance to those produced from protected, emasculated flowers, and flowers from which the stigma had been cut off in the bud. These pods are small and very often blue-coloured, seldom having the false septum completed, it being usually present as two little membranous frills down the inside edge of the pod. Thus it may be inferred that these swede (or turnip) × rape hybrids are quite without effect on cole types, the pollen not even exerting the stimulative effect on the fruit so commonly observed within the genus. That this lack of effect is not due to lack of viability of the various gametes is evidenced by the wonderful fertility of both pollen and ovules in compatible relationships.

Brussels sprout × kohl rabi. This hybrid produced much thickened stems, and at their apex a large number of buds similar to rather loose brussels sprouts. The line with brussels sprout as the pollen parent flowered both as an annual and as a biennial: the reciprocal flowered only in its first summer. The selfing results were as follows:

Method	Pods set. Flowers protected	Pods containing seed	No. of seeds in the fertile pods
Brussels sprout ♂ × kohl rabi ♀, 1st summer (annual)			
1	2 2 / 3 3	All	10
2	3 3 / 5 5	20	8
3	3 0 / 5 0	13	2
Same plant, 2nd year (biennial)			
1	2 3 / 5 1	0	—
2	4 3 / 5 8	19	1–12
3	4 0 / 5 0	0	—
Kohl rabi ♂ × brussels sprout ♀, 1st summer (annual)			
1	2 3 / 2 3	12	8
2	1 5 / 1 5	8	9
3	3 6 / 5 7	15	3–4

This again is a cross of self-sterile with a self-fertile parent, and two points of interest emerge from the results. In their first year both aspects of the cross are self-fertile. The plant, which persisted and flowered in its second year, was scarcely fully fertile and the fact that Method 1 produced no seed may be due to some disturbing factor which entered into the result, though none could be accounted for.

Purple sprouting broccoli × white heading broccoli. This cross is of peculiar interest as it involves what may be looked upon as two distinct types of sterility in one parent and a certain degree of fertility in the

other. White heading broccoli normally produces many more flower primordia than ever develop; a very large number abort, and are seen, when the inflorescence which develops from the edible head extends, as small brown withered knobs. Aborted primordia were not observed in the purple sprouting parent. Further, the white heading broccoli proved to be completely sterile in those flowers which did develop, but produced false pods as shown in Table I. Purple sprouting broccoli had been found to be comparatively fertile. The hybrid plants were
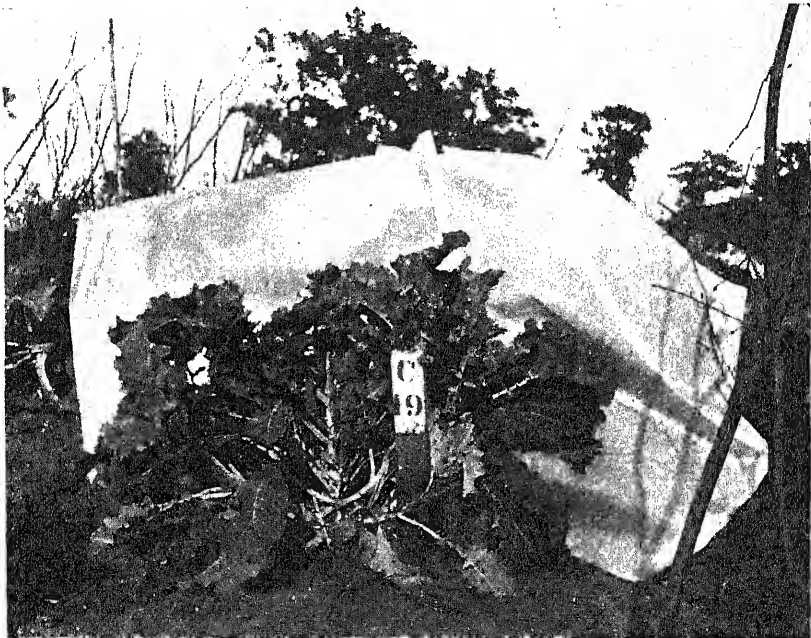
Fig. 1. F_1 plant of Purple sprouting broccoli × White heading broccoli.

extremely vigorous, vegetatively, throwing up a large number of side shoots and forming a bushy leafy plant as seen in Fig. 1[1]. Both aspects of the cross were identical in appearance. They did not flower in their first year, but produced a large number of purple broccoli heads in their second summer. A very large number of the flower primordia forming these heads aborted in the same way as the white heading parent did.

[1] The production of many side shoots in greater or lesser degree was characteristic of all those cole hybrids.

Not only were primordia shed, but buds in practically all stages of development were cut off. For those buds which developed the following results were obtained:

Method	Pods set. Flowers protected	Pods containing seed	No. of seeds in the fertile pods
Purple sprouting broccoli ♂ × white heading broccoli ♀, 2nd summer			
1	$\frac{48}{129}$	4	1–4
2	$\frac{39}{113}$	14	1–4
3	$\frac{0}{120}$	—	—
White heading broccoli ♂ × purple sprouting broccoli ♀, 2nd summer			
1	$\frac{0}{94}$	—	—
2	$\frac{0}{81}$	—	—
3	$\frac{0}{152}$	—	—

This hybrid seems to have received both forms of sterility with unimpaired intensity.

The turnip-swede-rape group. All the hybrids of this group sown in the autumn flowered during the following spring and summer. Hybrids involving turnip and swede, as has been mentioned, differ according to the way the cross is made. When turnip is used as the seed parent the resulting seed is weak, shrivelled and difficult to germinate. When swede is used as the seed parent the seed is plump and germinates strongly. Sutton (28), who reported this previously, found, however, that there was little or no difference in the hybrid plants once they became established. With the present writer, however, the hybrids which had had the swede for their seed parent were always rather stronger and more vigorous. They, too, produced more root buds[1]. Both aspects were affected by "finger and toe," the vigorous line producing larger "warts" but, when later on towards the end of the flowering period the plants were affected by mildew, the weak line was more affected than the aspect of the cross which had swede for seed parent (Fig. 2 shows typical plants). The plants, apart from vigour, were quite similar, and might briefly be described as white fleshed swedes with the white swedes' light-yellow-coloured flower, though the typical swede "neck" was very slightly developed. When selfed, neither aspect of the cross developed a single pod. Left to open pollination, to some extent isolated from other *Brassicae*, a large amount of seed was produced; this has been already noticed by Kajanus (18). It was observed in these studies that the line derived from the swede seed parent, though, if anything, farther removed from other compatible types, set rather more seed than its reciprocal, and it is assumed that some of this seed resulted

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[1] Kajanus (17) has discussed these malformations very fully.

from plant to plant pollination amongst the hybrids themselves. Various back and out crosses were attempted, and, as these later become somewhat involved, they are graphically represented in Figs. 3 to 7. In the figures the connecting lines join the forms crossed; the arrows indicate the direction the pollen was carried in; and the plus signs are estimates of the degree of fertility which obtained. The observations, from their very nature, could not be precise, and the difference between + + + and + + is not insisted on, but it is very probable that the difference

Fig. 2. Turnip swede hybrids. (C 21 had turnip as seed parent; C 22 had swede as seed parent.)

between + + + and + is quite significant. Plants of hybrid origin are shown as "fractions," the numerator always being the pollen parent. Thus $\frac{\text{swede}}{\text{turnip}}$ indicates a hybrid plant from the mating swede ♂ × turnip ♀. These back and out crosses were made to turnip, swede, rape, cabbage, mustard and radish. All the back crosses to turnip, swede and rape were more or less successful both ways. The out crosses to mustard and cole did not succeed in any case.

Distinct differences in fertility between the back crosses were noted. There is good evidence for stating that turnip pollen on the swede ♂
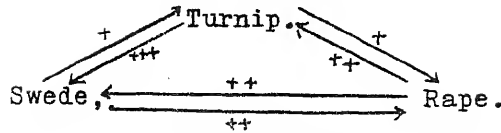
Fig. 3. Diagram to illustrate the fertility of inter-parental crosses.
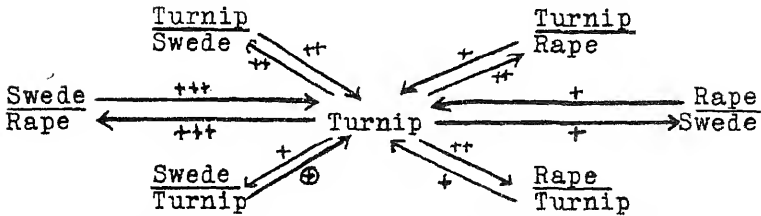
Fig. 4. Diagram to illustrate the fertility of crosses between hybrids and turnip.
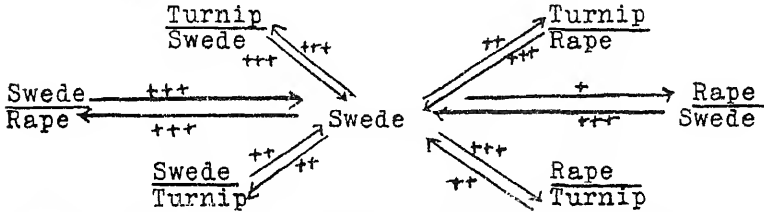
Fig. 5. Diagram to illustrate the fertility of crosses between hybrids and swede.
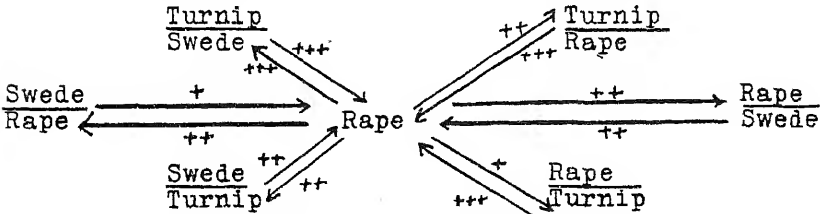
Fig. 6. Diagram to illustrate the fertility of crosses between hybrids and rape.
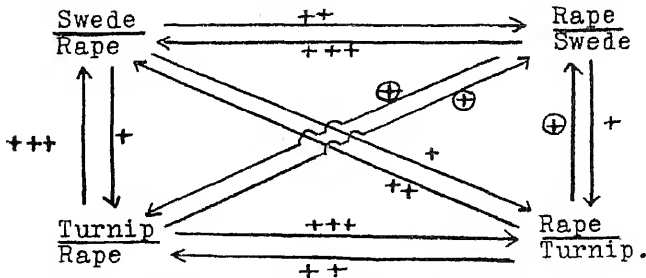
Fig. 7. Diagram to illustrate the crosses between hybrids.

× turnip ♀ hybrid is almost sterile; that swede pollen on the same hybrid is rather more effective; that turnip pollen on the turnip ♂ × swede ♀ hybrid is at least as effective; while swede pollen with this hybrid proved fully effective. The two aspects of the turnip × swede mating proved to be weakly fertile when crossed, but in the mating (turnip ♂ × swede ♀) ♂ × (swede ♂ × turnip ♀) ♀ the ovules aborted at a late stage and were incapable of germination. That is to say, turnip in the original cross proved to be a weak or poor seed parent, the progeny produced from it being as weak or weaker in a similar capacity, especially in response to turnip pollen. How far these differences of vigour in this mating are to be related to the mating of ♀ *Brassica oleracea* L. × ♂ *Raphanus sativus* L. which as reported by Karpetschenko [20] is fertile while its reciprocal is sterile, is difficult to determine. Are they both phases of the one underlying cause? Other crosses behaving similarly to the cabbage radish mating are known in other genera. Stout [25] notes these, and also that many grades exist in the comparative fertility of reciprocal crosses.

Considering crosses involving turnip with rape the mating of rape ♂ × turnip ♀ produced good viable seed; the opposite, however, gave weak shrivelled grains. When the F_1 plants were produced therefrom, there seemed to be a slight difference in vigour between the two aspects of the cross. The line having turnip as the seed parent was slightly more vigorous than the reciprocal. This is quite opposite to what was found in the turnip × swede matings, where the turnip seed parent produced weak seed which resulted in weak F_1 plants. It was noted, however, that all these turnip × rape hybrids were rather less vigorous than the pure rape (from the parent selfed) growing alongside. Both lots of hybrids produced a small root, only slightly thicker than that produced by rape itself. No root buds similar to those seen in the turnip × swede hybrids were observed. At flowering time they threw up a large number of flowering shoots from the "crown," producing a very bushy plant. A number of plants in all the rape hybrid cultures appeared to be affected with a disease somewhat similar in appearance to the "crinkle" which attacks potatoes. The plants used in these experiments were, so far as appearance went, normal in this respect.

Selfed, the mating turnip ♂ × rape ♀ did not produce a single pod by any of the three methods. The reciprocal (rape ♂ × turnip ♀) gave the following results:

Method	Pods set. Flowers protected	Pods containing seed	No. of seeds in the fertile pods
1	$\frac{17}{30}$	6	1–2
2	$\frac{48}{51}$	2	1–2
3	$\frac{0}{30}$	—	—

It is seen, then, that when the pollination was precise this aspect of the cross was to some extent fertile, and in its behaviour reminiscent of the turnip parent.

Back crossed to the parents and swede F_1 ex turnip ♂ × rape ♀ proved to be more or less completely fertile. With rape and swede there was some indication that the hybrid was more effective as a polleniser than as a seed parent. With turnip the fertility was rather lower. F_1 ex rape ♂ × turnip ♀, when back crossed, gave similar results.

Crossing these turnip/rape hybrids with swede/rape hybrids gave in the main very poor results, except that the mating of (turnip ♂ × rape ♀) ♂ × (swede ♂ × rape ♀) ♀ was highly successful, that is, when all the three "parental" types were involved and rape was the "common parent," being the *seed parent* of both of the plants. Further, it seemed clear in these matings of hybrids that turnip ♂ × rape ♀ was rather more successful than its reciprocal.

In crosses involving swede with rape it was noticed that the first parental cross was completely successful either way, both aspects producing abundant, plump and easily germinated seed.

The hybrid F_1 plants were in many ways similar to the F_1 plants from turnip × swede.

The roots were white fleshed and of fair size. No root buds were observed. A main flowering stem grew up and, though branched, there was no appearance of the many branches originating at the "crown," as was seen in the turnip × rape hybrids. The flowers were lemon-yellow like the rape parent. The F_1 resulting from rape ♂ × swede ♀ was somewhat weakly, due to heart rot. When selfed the two aspects gave the following results:

Methods	Pods set. Flowers protected	Pods containing seed	No. of seeds in the fertile pods
	Swede ♂ × rape ♀		
1	$\frac{25}{25}$	23	28
2	$\frac{16}{19}$	16	28
3	$\frac{28}{32}$	23	28
	Rape ♂ × swede ♀		
1	$\frac{35}{40}$	35	1–12
2	$\frac{28}{60}$	27	1–6
3	$\frac{28}{65}$	25	1–5

It may be mentioned in passing that Sutton's supposition that these hybrids would prove sterile is not well founded. The hybrid from swede ♂ × rape ♀ proved to be fully fertile with swede and turnip, but, curiously, rather less so with rape. The reciprocal (rape ♂ × swede ♀) was only fully fertile when used as the pollen parent on swede. With turnip it was only weakly fertile. The two aspects of the mating when crossed *inter se* were fully fertile. When crossed out to the turnip/rape hybrids the only mating which proved fully fertile was, as has been already shown, when the rape was the seed parent of both the hybrids mated.

DISCUSSION.

It is not proposed at this stage to develop any general discussion on fertility, but a few notes peculiar to the special point of view from which these studies are written will be offered.

Referring first to self-incompatibility, it seems clear that, in all the self-incompatible plants discussed, not of immediate hybrid origin, the cause of the incompatibility is the well-known inability of the pollen tubes to proceed through the stylar tissue. That is to say, the gametes are in all probability quite capable of uniting successfully but they never meet, the last stage of the male gametes' journey being interrupted. Whether this inability of the pollen to proceed through the stylar tissue is due to the production of an inhibiting substance, or to the non-production of some stimulating substance, was not decided by Correns (6), who discussed the phenomena in *Cardamine* and found evidence for the belief that they have a genetical basis involving a number of factor pairs which are transmitted according to a Mendelian scheme. Compton (5), who criticises the work of Correns, found evidence (4) in *Reseda odorata* for a simple scheme of Mendelian inheritance involving one factorial pair, self-fertility being dominant to sterility. Crane (7), working with plums and cherries, found no evidence to negative the view that self-sterility is a Mendelian recessive, but it appears that more than one factor is involved. East and Park (11) take the view that self-sterility is a condition determined by inheritance, but can be developed to its full perfection only in a suitable environment. The environment most suited to the development of self-sterility is stated by these workers to be one which promotes normal healthy vegetative growth during the active part of a flowering period. That self-sterile plants differ in their norms for self-sterility is also noted by East and Park. East and Mangelsdorf (12), working with species of *Nicotiana*, give a genetical explanation involving three factor pairs for the self-sterility results they report.

In the results reported here it is interesting to note that when cabbage (totally self-sterile) was crossed with purple sprouting broccoli (self-fertile) the F_1 was practically completely self-sterile. When, however, brussels sprout (self-sterile) which produced a completely self-sterile F_1 with the cabbage just mentioned was crossed with kohl rabi (self-fertile), the F_1 showed practically complete fertility as an annual, and only slight fertility in its second summer. What degree of fertility would have obtained if the plants had not flowered until their second summer with consequent unimpairment of the vigour of the vegetative phase is an interesting question. If it be assumed, however, that self-sterility is dominant in these coles, that brussels sprout was heterozygous and the cabbage homozygous, then a simple Mendelian explanation could be offered. The view adopted here, however, is that though self-fertility may be conditioned by inheritance, sterility, when it occurs, may, in practice, best be controlled by control of the environment rather than by the selection of self-fertile individuals.

The importance of "false podding" or fruitfulness accompanying sterility must not be overlooked. For the fruit grower this is obvious, but for the seedsman and plant breeder the question arises whether false podding may be looked upon as a promise of fertility when conditions are altered. Darwin [9] regarded so slight an effect as accelerated flower-fall following on pollination as a case of "incipient fertilisation."

The foregoing discussion which applies, as far as this report goes, only to parental types and F_1's within the cole group, does not apply to the crosses between species. It is practically certain that in the interspecific crosses absence of complete fertility, or the appearance of total sterility, has a cytological basis. Karpetschenko [19] has shown that all the forms examined by him belonging to the cole group (*Brassica oleracea* L.) have 18 chromosomes: the species to which the present writer believes his rape and, possibly, swede to belong (*Brassica napus* L.) has 36, while the species to which the turnip belongs (*Brassica rapa* L.) has 20. Brown mustard (B. *nigra* Koch.) has 16, while white mustard (*Sinapis alba* L.) has 24. The species of *Raphanus* have 18. All these numbers refer to the diploid phase. Regarding these results Karpetschenko concludes that the data of cytological investigation are also in conformity with the results of hybridisation: within the limits of separate genera plants having the same number of chromosomes are easily crossed, while the crossing of plants with different chromosome numbers is difficult. The further hybridisation results reported here agree, in the main, with this conclusion. All the coles are easy to cross and form a

natural group within a large species. The turnip-swede-rape group referred to here is not so homogeneous, for, viewed in the light of Karpetschenko's conclusion, and remembering Bailey's(2) opinion that swede has affinity with rape, the slight but clear differences between swede and rape in relation to turnip require some discussion. The swede and the rape probably do not occupy quite the same position phylogenetically; rather would it appear that rape in some way makes a slight approach towards turnip, or, possibly more correctly, the swede has diverged. The external appearance of the Essex' Broad-leaved Rape used in all these studies is very much like a white-fleshed swede which has failed to develop a bulb; both have the lemon-yellow turnip-like flower instead of the swede buff-coloured flower. The fact that rape shows some approach to compatibility with cole might be taken to indicate that it is the true *Brassica napus* L., having 36 chromosomes as reported by Karpetschenko, rather than swede, because the mating of 18 with 36 may be possible, and swede shows no indication of compatibility with cole, though here a physiological bar may be operating in addition. The cytological position in regard to the swede × turnip mating is unsatisfactory too, and it is likely that the fertility phenomena in connection with it reported here have a cytological basis. This supposition is supported by a parallel case in *Triticum*, reported by Watkins(29, 30), who crossed *Triticum turgidum* having 14 chromosomes with *T. vulgare*— 21 chromosomes[1]. He noted that all the grains resulting from the cross were shrunk and wrinkled, but those obtained when *vulgare* was the female parent were less so than the grains from the reciprocal cross. To note that in these *Triticum* and *Brassica* crosses, when the parent with the largest chromosome number was the female, the resulting seed was larger and more plump, is interesting and may be significant. It is to be remembered, of course, that the structure involved is a fruit in *Triticum* and a seed in *Brassica*.

While the vegetative weakness following on hybridisation in the turnip/swede cross, when the turnip was the female parent, may have the same cytological basis, the point requires confirmation. This reduction of vegetative vigour following on reduced fertility vigour and *vice versa* can hardly be related to the decrease in fertility vigour following on decrease of vegetative vigour seen in so many other cases. What is implied here by "reduced vegetative vigour" is illustrated in Pl. IX, figs. 1 and 2. The differences between the lots are striking in view of the fact that all the plants were sown on the same day and

[1] Haploid numbers.

every possible precaution taken that they should all receive the same treatment.

The results of selfing by Method 3 (isolation of the spike from every type of pollinating agent) may be briefly mentioned. They are offered here simply as an indication of the necessity for deliberate pollination in order to obtain selfed seed of certain plants. The low yield of pods and, in the case of self-compatible plants, of seeds may be ascribed entirely to an insufficiency of pollen reaching the stigma. This inability to self-pollinate, which, in many cases, undoubtedly results from spacial and time relationships of flowers and flower parts, will be discussed elsewhere.

SUMMARY.

1. The paper deals with the cross- and self-fertility of certain forms of the genus *Brassica*.

2. It is shown that "rogue" production between two commercial crops depends on the amount of pollen of one incident on the aggregate stigmatic area of the other and a "constant" applicable to the relationship under consideration.

3. The "constant" is shown to be the resultant of the self-compatibility of the one crop and the cross-compatibility which exists between the two.

4. The value of the two components of the "constant" is shown to depend upon, and largely to be controlled by, the environment.

5. Relationships involving various *Brassicae* which might lead to valuable recombinations are discussed.

6. Weakened vegetative vigour following on weak fertility vigour and *vice versa* are noted and discussed.

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Fig. 1.

Fig. 2.

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EXPLANATION OF PLATE IX.

Fig. 1. On the right are shown two rows of turnip ♂ × (swede ♂ × turnip ♀) ♀ and next to them on the left two rows of rape ♂ × (swede ♂ × turnip ♀) ♀.

Fig. 2. Reading from right to left are shown:—(turnip ♂ × swede ♀) ♂ × rape ♀ turnip ♂ × (turnip ♂ × swede ♀) ♀ (47); (swede ♂ × turnip ♀) ♂ × turnip ♀ (48); swede ♂ × (swede ♂ × turnip ♀) ♀ (49); (swede ♂ × turnip ♀) ♂ × swede ♀ (50).

THE INFLUENCE OF THE "PURPLE" GENE ON THE CROSSING-OVER BETWEEN "BLACK" AND "CINNABAR" IN *DROSOPHILA MELANOGASTER*

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CONTENTS.

INTRODUCTION.

THE following contribution from the Anikovo Genetical Station is a collective one in which the most active part was carried out by students: viz. W. W. Sacharov (in 1923–24), O. A. Ivanova, L. N. Promptova and L. W. Ferry (1924). It was only possible to carry out the important part of this work, *i.e.* the hatching and counting of 120,000 flies in the comparatively short time of July–September 1924, thanks to the assiduous work of the above-named persons.

We used cultures of *Drosophila melanogaster* brought to the Institute of Experimental Biology in 1922 by Prof. H. J. Muller of Texas University. We availed ourselves of his kind advice in the organisation of the experiment and in the working out of the plan. In the winter of 1923–24 he also sent us from America a special culture of *Drosophila*, which unfortunately arrived frozen, and a second parcel sent in spring never reached us, though we do not know the reason. While regretting our double misfortune we wish to avail ourselves of this opportunity of expressing our gratitude to Prof. Muller, for, thanks to him, Russian

scientists are now able to work with such an exceptional object as *Drosophila melanogaster* and its 30 best mutations.

We express our gratitude also to Prof. B. S. Jastrembsky and Prof. N. S. Chetverikoff whose advice has greatly helped us in the mathematical part of our work.

In the first part of our paper we shall examine the material used in our attempt at measuring the size of the recessive mutation "purple." The second part is devoted to an analysis of the variation and distribution of the rare cross-over.

The question of the nature of the gene and of its physical and morphological qualities is one of the important questions in genetics, but up to the present we can give no definite answer to it. We know that the gene is more or less definitely localised in a certain chromosome, and in a defined part of it, remaining there during the crossing-over, and sometimes participating in the duplication or translocation of this chromosome. We can consider the gene as a part of a chromosome, and, as such, possessing a definite space extension. We can derive the same conclusion from the known proportion between the number of genes in a given chromosome and the size of the chromosome itself. From the repetition of some mutations Morgan and others tried to find out the size and number of the genes of *Drosophila*, and came to the conclusion that *Drosophila melanogaster* has in its haploid set of chromosomes possibly about 2000 genes. Owing to the fact that the combined length of the four chromosomes is equal to $7 \cdot 5 \mu$ Morgan defines the size of the gene, only approximately of course, as being $0 \cdot 02$–$0 \cdot 06$ mikron. But the theoretical basis of that calculation proved to be insufficient. Nowadays we consider that repeated mutations cannot be explained by random sampling according to the laws of chance, but that they are called forth by a heightened liability in particular parts of a chromosome. The $1 \cdot 7$ morganid of the sexual chromosome is certainly one of such parts where mutations occur exceptionally often. In illustration we may mention the fact that the *Drosophila* brought from America to our laboratory gave its first mutation (white) at that part. The *Drosophila* brought from Crimea also gave white eyes as a first mutation; this last mutation was not experimentally investigated, the mutant flies having perished, but the identity of the genes in all these cases is very probable. We may add that the related species of *Drosophila* also show a similar liability to mutation at a homologous point on the *X*-chromosome.

But if repeated mutations owe their origin to a special liability at certain points in the chromosomes we are no more able to judge of the

number of genes, and consequently the possibility of defining the size of each gene by a simple arithmetical process disappears. To determine the size of the gene we must look for some other method.

We can for instance approach the problem of measuring the length of genes (in morganids) by the method of comparisons. Thus the American genetists have no doubt that "deficiency" can be measured, and Bridges has effected the measurement of Bar deficiency for the first time. Deficiency embracing at once an area occupied by several genes (for instance Notch) immediately shows its dimension.

The detailed study of Notch deficiency enabled Mohr to conclude that we cannot trace any difference between deficiency and dominant mutations. Both are accompanied by a recessive lethal effect of other genes. Mohr gave a pretty explanation of the fact, which at first seems a little strange, that the dominant character is called forth by the falling out (or inactivity) of part of a chromosome. For example, let genes A, B, C influence the given character in one direction and genes M, N, O in the reverse direction. We can then symbolise a certain known normal position of a character X by the expression:

$$X = \frac{AA \quad BB \quad CC \ldots\ldots\ldots}{MM \quad NN \quad OO \ldots\ldots\ldots}$$

Now let one part of a chromosome, containing A, fall out (be inactive), transforming itself into a. It is evident that the balance will be disturbed and the character X will be changed into X', the "recessive" change leading to a dominant effect. This of course can only occur in the case where the remaining allelomorph A is unable fully to compensate the effect of its inactive partner a.

Hence we see that dominant mutations can be explained in two ways. We can explain them *a priori* as a result of the apparition of a new element in the chromosome. Then the dominant mutations will show a contrast to the recessive ones in the following order:

(a) Dominant mutation: the apparition of a new element in the chromosome.

(b) Normal condition: chromosome without change.

(c) Recessive mutation: some elements disappear in the chromosome.

(d) Deficiency: some elements disappear in the chromosome.

From another point of view we may also consider dominant mutations as stronger mutations, but with the same disappearance of elements in the chromosome.

(a) Normal condition: chromosome without change.

(*b*) Recessive mutation: falling out of an element in the chromosome; but the remaining allelomorph proves sufficient to prevent the external manifestation of the mutation (case of complete dominance).

(*c*) Strong recessive mutation: falling out of a stronger part, owing to which the remaining allelomorph is not capable of fully suppressing the manifestation (case of incomplete dominance).

(*d*) Dominant mutation: the element of the chromosome which fell out is quite insufficient, and the effect of mutation is clearly to be seen.

(*e*) Deficiency: the element of chromosome which fell out is still larger.

In *Drosophila* we have typical recessive mutations imperceptible in the heterozygous condition, "strong recessive mutations" which are perceptible in the heterozygous condition (ebony, black, etc.) and, dominant mutations which are mostly so "strong" that they prove in the homozygous condition to be quite unfit for life[1].

We consider it probable that each recessive mutation can be considered as a "little deficiency," and we are confirmed in our conviction by the fact that many of them simultaneously affect two or more characters, which are apparently not at all connected one with another, *e.g.* coloration and bristles, wings and eyes, etc.; and we can easily form a continuous series beginning with mutations having evidently only one symptom and ending with those which, as typical deficiencies, have several manifestations.

But if in principle we admit the absence of difference between deficiency and mutations, we must agree that the method often used for defining the length of deficiency can be used also to define the length of the gene. Hence, by choosing three adjacent genes and by studying the crossing-over between the two extreme ones we should be able to determine the size of the middle one. Our work represents such an attempt.

Gowen in 1919 definitely formulated this interesting problem. "If the gene is not an integral part of the mechanism of heredity, but only connected with it, we should not expect its size to affect the crossing-over. But if allelomorphs are definite physical granules in the chromosomes, and crossing-over occurs as a result of a breaking of the thin twisted

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[1] From this arises the question whether there may not exist two types of dominant mutation, one with formation of new elements in the chromosome, the other with falling out of strong elements. The differences in the viability of different mutations in *Drosophila* seem to suggest it; for some dominant mutations are in the homozygous condition non-viable, others quite the contrary.

threads, we should expect the substitution of one type of granule for another to have a certain influence on the points and number of the breakages of the chromosomes; just as the breaking of a metallic cable in a given place will depend upon what kind of metal, whether silver or steel, the given point is made of" (216).

But the facts concerning the influence on the crossing-over of substitution of some genes by others which were collected by him cannot be used by us, because his problem concerned asymmetrical structures or the difference between the symmetrical and asymmetrical ones. Unfortunately in his Table IX, the most interesting for us, are evidently two misprints. According to Table IX, Table X must be read thus:

Formula of the reversed crossing	Abbreviated formula	Without crossing-over	Crossing-over		
			1	2	3
$A\ \dfrac{s_a\,s_s\,c^s\,r_o}{D+}$	$s_a\,s_s\,c^s\,r_o$	17463	6015	3330	6400
$B\ \dfrac{s_e\,s_s\,c^s\,r_o}{+H}$	$s_e\,s_o\,c^s\,r_o$	1469	510	249	543

or in per cent.

		0	1	2	3
A	A	55·6	19·2	10·6	20·3
	B	55·8	19·4	9·5	20·7

PART I. METHODS AND MATERIAL.

As mentioned above, the cultures of *Drosophila melanogaster* brought from America by Prof. Muller in 1922 were used as material for our investigation. Among other mutations he brought "black" (black colouring of body), "purple" (purple eyes) and "cinnabar" (cinnabar eyes). These three mutations are localised in the middle part of chromosome II sufficiently near one another. According to the latest plan of this chromosome, as determined by Bridges, black is localised on morganid 47·5, purple on 53 and cinnabar on 54. Unfortunately we have at our disposition no other triplet of good mutations localised so near one another.

The region of chromosome II that we chose proved to be not quite satisfactory owing to the great dependence of crossing-over in that region upon different internal and external influences; this fact was mentioned by Plough in his work on the influence of temperature on crossing-over. Later on, during the course of our work, this point of view was confirmed by the work of Mavor and by our own investigations.

But even to-day we have no other chromosomal region which is more satisfactory, for the proximity of the genes we are studying is of the

greatest importance. The modification in the size of crossing-over that could have occurred during our investigation must have been very small, and during the investigation of mutations remotely situated one from another ought to have disappeared behind casual variations of crossing-over. Thus we see that even *Drosophila melanogaster*, widely investigated as it is, does not give us sufficient choice of suitable material for the solution of our problem, especially in Russian laboratories. We mention this to show that further searches for new mutations in this fly might possess great importance for the further investigation of the mechanics of inheritance.

In order to observe the crossing-over between these three mutations it was necessary to obtain the line of "recessive analysators" with three recessives (black—purple—cinnabar). This was very difficult owing to the close proximity of purple and cinnabar, and was only accomplished towards spring 1924 (W. W. Sacharov). (1) The line II *pl.* (dumpy, black, purple, curved, plexus, spectre), (2) the line black vestigial, and (3) the line cinnabar served as the pure lines for its making.

In the line thus obtained we included also the mutation "dumpy" (shortened wings) which is localised far from the other three, viz. on morganid 9. The rôle of this mutation was to prove that during the investigation there did not occur any unexpected internal or external changes that could influence the size of the crossing-over in the whole chromosome II.

So we obtained the line dumpy-black-purple-cinnabar $(T^d b p_r c_n)$. But naturally there arose doubts as to what degree it was possible to consider this line "pure," or absolutely homozygous. Besides visible mutations, many mutations are known which are either "on the limit of perceptibility" or quite imperceptible. To the last belongs the large category of "intensifiers" that manifest themselves only in the presence of the mutation whose effect they strengthen. Thus notwithstanding our having a homozygous line in respect of the four mutations mentioned there is always a chance that between them are localised some imperceivable mutations for which this line might be heterozygous. If we take flies for the investigation of crossing-over from such a line it is clear that these flies may sometimes be homozygous, and at other times heterozygous in respect of invisible mutations. In connection with the interpretation of crossing-over discussed this circumstance would essentially alter the result.

To avoid this possible source of failure, we obtained a line which possessed all the II chromosomes absolutely *identical* by using the

following method. We had at our disposal the dominant mutation *Curly*
(with wings curled upwards) in the line $\dfrac{C_y \quad c_n}{St \; T^d \, b \; p_r}$ which if localised in
the left part of chromosome II closes the crossing-over in the whole
length of the chromosome.

In the Curly line, which cannot exist in the homozygous state, another
dominating gene had been localised in the other second chromosome,
which is also non-viable in the homozygous state, viz. *Star* (rough eyes),
together with mutations *dumpy*, *black* and *purple*.

The hybrids with $T^d\, b\, p_r\, c_n$ had the structure

$$\frac{C_y \qquad\quad c_n}{T^d\, b\; p_r\, c_n}.$$

One of such flies was again recrossed with the *Curly* line. Since
homozygous Curly is non-viable the crossing follows this scheme.

$$\frac{C_y \qquad c_n}{T^d\, b\; p_r\, c_n} \times \frac{C_y \qquad c_n}{S\; T^d\, b\; p_r}:$$

1. $\dfrac{C_y \quad c_n}{C_y \quad c_n}.$ 3. $\dfrac{T^d\, b\; p_r\, c_n}{C_y \qquad c_n}.$

2. $\dfrac{C_y \qquad c_n}{S\; T^d\, b\; p_r}.$ 4. $\dfrac{T^d\, b\; p_r\, c_n}{S\; T^d\, b\; p_r}.$

Thanks to the absence of crossing-over in this cross we get only four
theoretical combinations, as is shown in the scheme. Since one is non-
viable there remain only three combinations. Two of them have wings
turned upwards (Curly), and one differs markedly from the others owing
to the outwardly visible mutations dumpy, black, purple. The first two
differ one from another, especially in the colouring of their eyes, which is
either normal or cinnabar. Choosing flies with cinnabar eyes and wings
turned upwards we shall have to deal only with the combination

$$\frac{C_y \qquad c_n}{T^d\, b\; p_r\, c_n}.$$

All these flies have the chromosome *dumpy*, *black*, *purple*, *cinnabar*
absolutely identical, or at any rate are derived from one chromosome of
the mother fly without any change by crossing-over. Owing to the absence
of crossing-over (closed by Curly gene) we are able to get the following
segregation by crossing two such flies:

1. $\dfrac{C_y \qquad c_n}{C_y \qquad c_n}.$ 3. $\dfrac{T^d\, b\; p_r\, c_n}{C_y \qquad c_n}.$

2. $\dfrac{C_y \qquad c_n}{T^d\, b\; p_r\, c_n}.$ 4. $\dfrac{T^d\, b\; p_r\, c_n}{T^d\, b\; p_r\, c_n}.$

The fourth combination is an ideal-pure line of course only in relation to chromosome II. We did not trouble about the purity of the other chromosomes, because nothing that we know about the inhibitory genes suggests that the possible presence of an invisible mutation in some other chromosome would influence the crossing-over between black and cinnabar.

This line of analysators enabled us to find some other useful lines. We divided our experiments into four parts. Crossing-over was investigated:

(1) In females having the structure $\dfrac{-}{b\ c_n}$ (we will call this structure the PP compound).

(2) Females of structure $\dfrac{p_r}{b\ p_r\ c_n}$ (pp compound) in which the normal allelomorphs in both II chromosomes were replaced by the recessive allelomorphs pp.

(3) Females of structure $\dfrac{-}{b\ p_r\ c_n}$ (Pp compound) in which the females had different chromosomes; from our point of view their length was different, the difference being equal to the supposed length of gene P.

(4) Females of structure $\dfrac{c_n}{b\ p_r\ c_n}$ (cc compound).

This last case differs from the preceding one by modifications in the structure in the part of cinnabar.

Our argument was as follows. If the substitution of the normal allelomorph by the recessive one shortens the chromosome by the length of the gene taken out, then the crossing-over between black and cinnabar in the second case (pp compound) will also appear reduced in comparison to the first case (PP compound). But this reduction could be also called forth by the fact that the physiology of pp flies is a little different from that of normal flies, and therefore the crossing-over in these flies might be somewhat modified. The cross of the third type (Pp compound) serves for further verification. These flies are phenotypically like the PP flies, and if modifications in the crossing-over are induced through the influence of mutation purple on the physiology of the flies, then the crossing-over in PP flies and in Pp flies will be alike, or almost alike (if P does not absolutely dominate p). But from our point of view other reactions may happen here, for a Pp fly may exhibit asymmetry of structure in its II chromosomes, and crossing-over may in consequence be more difficult than in the pp fly.

On the other hand, if we regard the conditions of crossing-over in flies of class (3) as compared with class (4) and pay attention to the crossing-over between black and purple, we shall see that from our point of view the conditions are alike in both cases. For the difference between cases (3) and (4), namely the substitution of $C_n c_n$ for $c_n c_n$, takes place outside the black-purple region. Concerning the general modifications in the conditions of the crossing-over with cinnabar flies, we can say that in comparison to normal flies the difference between cases (3) and (4) is the same as between cases (1) and (2), the probability of modifications in the size of crossing-over being equal in both pairs.

Flies needed for those experiments were obtained by the following ways: The experiment began by the crossing of a normal fly with a male-analysator, and the hybrids were always crossed with males of the pure line of analysators. In the back-cross of hybrid females with analysators there arose, thanks to the crossing-over, different new types, out of which it was easy to combine females with different structure. We never used any other flies for our crossings, and therefore all the modifications in the genotype could have happened only from mutations.

We can state therefore that the material used for our experiments was of sufficient purity and uniformity. Our second serious care was for the uniformity of environmental conditions. We know that crossing-over can be strongly modified according to the conditions. In the first place we must mention temperature having, as Plough has established, a great influence on the crossing-over. Therefore we carried out our experiment in a thermostat. Unfortunately the scanty equipment of the laboratory of the Anikovo station disturbed our work, especially in July when sometimes it was quite impossible to keep the temperature at a level of 25°. Nevertheless, we consider that the fluctuation of temperature which thus occurred had no essential effect on the result of the experiment. From Plough's investigations we know that the curve which marks the dependence of crossing-over on the temperature takes a characteristic course, modifications of temperature between 17° and 29° having a very little effect on the crossing-over, only further modifications of temperature out of these limits producing great changes; therefore we chose the temperature of 25° as one in which small modifications would have the least effect on the crossing-over.

It was also necessary to consider the age of the females, for it has been established by many investigators that the females' age affects the crossing-over. In order to exclude this cause of variations we have put each newly hatched female and male in a test-glass marked " A "

and after six days in another marked "B." On the twelfth day we threw out the female; thus we obtained two batches of eggs laid by her, viz. "A" eggs laid during the first six days of her life, and "B" eggs laid during the second period of six days. As will be noticed later in the description of our results, the amount of crossing-over in these two experiments is so different that we must examine them separately.

Lastly we directed our attention to the size of the females as a possible cause of variations in the crossing-over. During the first days of emergence the newly hatched flies are large, then day by day, they become smaller, and in 15–20 days they emerge as dwarfs. Evidently humidity here plays the chief rôle, and the late emerging flies may perhaps be regarded as more "concentrated," which may of course affect the crossing-over. Thus we marked for each female the day of her hatching, reckoning from the day when the hatching began in this test-glass. In this way we obtained "females of day I," "females of day II," etc. to "females of XX day."

We counted every day in each test-glass the newly hatched flies (sometimes twice) during fifteen days, after which we cleaned out this test-glass, from fear of contamination by the next generation. Our records are represented on Table XIV by four examples.

TABLE I.

PP compound, *A* cultures.

		Day of emergence									
Constitution of ♀♀		I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\dfrac{c_n}{T^d\,b}$	Number of flies, $-n$	1008	795	1052	488	872	496	596	541	161	373
	% crossover on the 1st region	31·7	29·2	26·9	29·1	28·8	27·4	25·4	25·9	30·4	22·8
	% crossover on the 2nd region	7·0	8·4	5·4	5·7	3·4	3·6	4·7	2·9	1·9	6·4
2. $\dfrac{}{T^d\,b\,c_n}$	n	413	529	538	415	404	382	489	—	73	—
	%, 1	33·7	28·2	24·5	28·7	26·0	27·7	25·6	—	17·8	—
	%, 2	4·8	10·0	6·1	4·8	7·4	5·0	5·3	—	2·7	—
3. $\dfrac{T^d\ \ c_n}{b}$	n	132	81	111	—	—	—	—	—	108	—
	%, 1	32·7	23·6	27·0	—	—	—	—	—	34·3	—
	%, 2	9·1	3·6	1·8	—	—	—	—	—	1·8	—
4. $\dfrac{T^d}{b\ c_n}$	n	368	64	—	—	—	—	—	104	—	—
	%, 1	31·0	34·4	—	—	—	—	—	20·2	—	—
	%, 2	6·5	6·2	—	—	—	—	—	12·5	—	—

TABLE II.

PP compound, *B* cultures.

Constitution of ♀♀		Day of emergence									
		I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\dfrac{c_n}{T^d\,b}$	n	658	634	1134	344	559	586	373	176	174	383
	%, 1	24·5	24·9	26·4	27·3	22·2	25·4	24·1	21·6	27·6	21·4
	%, 2	3·3	3·6	2·3	4·1	2·3	3·6	2·9	0·6	1·1	3·1
2. $T^d\,b\,c_n$	n	256	457	485	167	448	290	232	—	—	—
	%, 1	28·5	26·0	27·2	27·0	29·0	25·5	25·9	—	—	—
	%, 2	4·3	4·6	1·6	1·8	3·3	4·5	3·9	—	—	—
3. $\dfrac{T^d\quad c_n}{b}$	n	57	75	76	—	129	—	56	—	124	—
	%, 1	31·6	18·7	34·2	—	25·6	—	26·8	—	37·1	—
	%, 2	1·7	2·7	1·9	—	4·6	—	3·6	—	2·1	—
4. $\dfrac{T^d}{b\,c_n}$	n	243	148	76	—	—	—	—	—	—	—
	%, 1	32·1	20·3	25·0	—	—	—	—	—	—	—
	%, 2	3·7	4·0	7·9	—	—	—	—	—	—	—

TABLE III.

pp compound, *A* cultures.

Constitution of ♀♀		Day of emergence									
		I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\dfrac{T^d\,b\,p_r}{p_r\,c_n}$	n	1155	411	694	537	671	570	252	333	89	434
	%, 1	29·5	32·3	29·7	26·6	25·4	29·1	27·0	25·5	18·0	26·7
	%, 2	4·5	6·3	3·7	3·7	2·7	2·6	3·6	1·8	6·7	3·9
2. $\dfrac{p_r}{T^d\,b\,p_r\,c_n}$	n	951	653	1183	1007	596	433	541	52	337	94
	%, 1	23·5	27·9	28·4	25·8	24·5	24·7	26·4	21·4	22·2	28·7
	%, 2	4·6	5·5	6·1	4·7	5·5	3·7	3·7	3·8	3·0	5·3
3. $\dfrac{T^d\quad p_r\,c_n}{b\,p_r}$	n	156	180	—	43	146	188	234	152	—	—
	%, 1	32·0	28·9	—	39·5	32·9	30·3	32·5	27·6	—	—
	%, 2	8·3	6·1	—	14·0	5·5	10·6	6·8	2·0	—	—

TABLE IV.

pp compound, *B* cultures.

Constitution of ♀♀		Day of emergence									
		I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\dfrac{T^d\,b\,p_r}{p_r\,c_n}$	n	736	774	418	470	400	385	216	290	—	507
	%, 1	22·7	24·8	22·5	22·1	25·7	22·6	16·2	22·4	—	26·0
	%, 2	3·8	4·3	1·4	2·8	3·0	2·5	4	5·7	—	4·3
2. $\dfrac{p_r}{T^d\,b\,p_r\,c_n}$	n	831	1004	937	548	496	575	—	—	252	252
	%, 1	22·6	25·2	24·0	23·6	25·6	22·4	—	—	22·6	21·4
	%, 2	3·3	3·0	2·6	2·2	2·9	1·0	—	—	2·0	2·0
3. $\dfrac{T^d\quad p_r\,c_n}{b\,p_r}$	n	112	121	—	—	143	189	—	85	—	—
	%, 1	31·2	25·6	—	—	24·5	30·1	—	30·6	—	—
	%, 2	2·7	3·3	—	—	5·6	7·4	—	2·3	—	—

TABLE V.

Pp compound, *A* cultures.

Constitution of ♀♀			I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\overline{T^d\,b\,p_r\,c_n}$		n	6994	6837	4419	3375	1478	2092	1210	560	420	346
		%, 1	28·8	28·4	28·5	29·0	25·4	28·1	27·8	25·0	25·2	26·3
		%, 2+3	4·2	4·5	3·3	3·8	3·7	2·8	3·3	2·5	1·4	2·6
2. $\dfrac{T^d\,b}{p_r\,c_n}$		n	70	388	—	147	203	72	—	—	—	—
		%, 1	40·0	32·2	—	25·9	23·4	29·2	—	—	—	—
		%, 2+3	7·1	10·3	—	8·2	10·3	4·2	—	—	—	—
3. $\dfrac{T^d\quad c_n}{b\,p_r}$		n	184	248	264	134	72	123	95	87	65	91
		%, 1	35·9	30·4	32·6	30·6	25·0	26·8	26·3	41·4	20·0	23·4
		%, 2+3	6·5	6·6	4·2	2·2	2·8	1·6	2·1	2·3	3·4	0·0

TABLE VI.

Pp compound, *B* cultures.

Constitution of ♀♀			I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\overline{T^d\,b\,p_r\,c_n}$		n	4971	3955	3414	2575	964	1328	401	437	80	255
		%, 1	25·4	26·6	25·5	25·5	23·4	23·6	18·4	24·0	18·7	29·0
		%, 2+3	2·9	2·3	2·0	2·3	2·3	2·5	1·2	1·4	1·2	2·0
2. $\dfrac{T^d\,b}{p_r\,c_n}$		n	281	340	—	91	205	—	—	—	—	—
		%, 1	28·5	32·3	—	38·5	21·5	—	—	—	—	—
		%, 2+3	3·91	2·6	—	5·5	7·8	—	—	—	—	—
3. $\dfrac{T_p\quad c_n}{b\,p_r}$		n	53	205	305	114	60	57	—	43	—	111
		%, 1	30·2	34·7	25·9	23·7	25·0	26·3	—	37·2	—	21·7
		%, 2+3	1·9	3·4	4·3	0·9	3·3	1·7	—	4·6	—	2·7

TABLES VII and VIII.

cc compound, *A* cultures.

Constitution of ♀♀			I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\dfrac{c_n}{T^d\,b\,p_r\,c_n}$		n	1344	1276	608	589	406	275	653	1230	84	550
		%, 1	26·7	26·3	32·4	28·5	26·8	34·5	28·0	26·3	19·0	23·8
		%, 2	4·2	2·7	6·7	4·4	3·4	4·0	2·6	3·5	1·2	1·8

B cultures.

			I	II	III	IV	V	VI	VII	VIII	IX	X
1. $\dfrac{c_n}{T^d\,b\,p_r\,c_n}$		n	1219	834	558	690	201	72	337	820	80	246
		%, 1	28·4	25·4	26·5	29·4	22·9	29·2	23·4	25·4	20·0	30·5
		%, 2	1·8	1·6	2·5	2·9	2·0	5·5	1·5	2·3	3·7	1·7

In Tables I–VIII we give the final results of the experiments on the structure of the females, the types of cultivations (*A* and *B*), and the days of the hatching of the females; while the absolute numbers of flies are also given as percentages of the crossing-overs in the two regions, from T^d to b (the row: %, 1) and from b to c_n (the rows: %, 2 or %, 2 + 3).

We have not given a general summing up of the types of compounds. If, as we can see from the results of our experiments, the recessive mutation is the shortening of a chromosome, then it is not evident that such structures for example as $\dfrac{}{b\ p_r\ c_n}$ and $\dfrac{c_n}{b\ p_r}$ could give equal crossing-over. With the accumulation of all recessives in one chromosome the asymmetry will evidently be stronger than with a proportional distribution of recessives between the two homologous chromosomes which enter into the crossing-over. Unfortunately this question cannot be solved by the usual method of studying the crossing-over, since we must consider the different viability of the classes of cross-overs obtained. In order to explain this we must use Muller's methods (back-cross with normal flies).

THE RESULTS OF THE EXPERIMENTS.

The influence of "the day of emergence" on the amount of crossing-over. In the description of our methods of work, we mentioned that we marked for each female the day on which she emerged after the beginning of emergence in the given culture. The largest flies emerged during the first days, while flies emerging on the 15th–20th in the conditions of our experiment were but half the size of the first ones.

We do not of course consider that chromosomes grow smaller with a decrease in the size of the flies. As is well known, variation in size is dependent only on the variation of the number of cells in the body. Since the diminution of size in the flies is apparently a function of the drying up of the medium in which they live, we can assume that the bodies of the later flies will be drier and more concentrated, which may in its turn affect the viscosity of the plasma and also the conditions in which the process of crossing-over takes place.

The results obtained by us show a diminution in the value of the crossing-over in flies of the last days, in both categories A and B, and in all points of crossing-over. We can see this general diminution in the numbers of the two % rows in Tables I–VIII. When we observe in each female a complete parallelism between the corresponding diminution of size of the crossing-over and its growth, the causes in both cases must in our opinion be the same.

Further on we shall mention the theoretical significance of this fact. But for the present we are more interested in the methodological side of the question, the importance of these changes for the calculation of the distances between the genes being evident.

Indeed, if the amount of crossing-over is changed in flies hatched on different days, then if we are using for our experiment such flies we certainly shall obtain essentially different "relative distances." In the investigation of a small region this influence can be so great that the "relative distances" may differ in scores of percentages, which is of great importance for our theme. To choose an average quantity for different days is evidently vain, for this average will only characterise the amount of crossing-over on some casual "average day," and cannot be compared in different experiments, inasmuch as the days of emergence of females were not marked by the experimenters.

In order to eliminate the influence produced by "day of emergence" we ought to discover the law according to which the amount of crossing-over varies, and when the equation corresponding to this law is found we must deduce from it the influence of the day. In our case this problem is much simplified, for the diminution is represented quite satisfactorily by the linear dependence, at least for the first ten days of the experiment. In the cases where the number of experiments is large enough (see the structure $\overline{T^d\ b\ p_r\ c_n}$ A and B) the linear character of dependence is quite evident, at least for the practical purpose of introducing corrections in "the day of hatching."

TABLE IX.

The Linear Equations expressing the connection between the size of Crossing-over and the days of Hatching of the Females investigated[1].

Structures	A cultures	B cultures
1. $\overline{T^d\ b\ p_r\ c_n}$	$y_1 = -0.28x + 28.78$ $y_2 = -0.20x + 4.53 \pm 0.12$	$y_1 = -0.54x + 27.10$ $y_2 = -0.10x + 2.80 \pm 0.15$
2. $\overline{T^d\ b\ p_r\ c_n}^{\ p_r}$	$y_1 = -0.21x + 26.25$ $y_2 = -0.21x + 5.68 \pm 0.22$	$y_1 = -0.09x + 23.24$ $y_2 = -0.17x + 3.14 \pm 0.20$
$\overline{T^d\ b\quad c_n}$	$y_1 = -0.60x + 29.01$ $y_2 = -0.24x + 7.16 \pm 0.52$	$y_1 = -0.50x + 29.41$ $y_2 = -0.04x + 3.74 \pm 0.49$
$\overline{T^d\ b\ p_r\ c_n}^{\ c_n}$	$y_1 = -0.55x + 32.51$ $y_2 = -0.12x + 5.01 \pm 0.28$	$y_1 = 0.08x + 25.9$ $y_2 = 0.02x + 2.70 \pm 0.24$
5. $\overline{T^d\ b}^{\ c_n}$	$y_1 = -0.64x + 30.96$ $y_2 = -0.44x + 7.43 \pm 0.20$	$y_1 = -0.06x + 24.8$ $y_2 = -0.15x + 3.49 \pm 0.20$
6. $\overline{T^d\ b\ p_r}^{\ p_r\ c_n}$	$y_1 = -0.73x + 31.32$ $y_2 = -0.11x + 4.34 \pm 0.24$	$y_1 = 0.15x + 23.20$ $y_2 = 0.06x + 3.41 \pm 0.32$

$y_1 =$ crossing-over between T^d and b,
$y_2 =$ crossing-over between b and c_n,
$x =$ day of emergence of the females investigated.

[1] Calculated by O. A. Ivanova. With constant terms of equation (2) their standard error.

The equations of all the lines are given in Table IX.

These equations have two quantities that are defined with the standard error, the tangent of the angle of inclination, and the constant C. Now there arises the question, as to which size of the crossing-over, which sense of y we must choose as total summary of the experiment. We think the more applicable sense of y will be x being equal to 0, as in this case

TABLE X.

The Total General of Experiments.

The amount of crossing-over in females of different constitution which all emerged on the zero day.

| Constitution of females | Value of crossing-over | | | |
| | between b and c_n | | between T^d and b | |
	A	B	A	B
PP	$7 \cdot 21 \pm 0 \cdot 29$	$3 \cdot 68 \pm 0 \cdot 22$	$30 \cdot 99 \pm 0 \cdot 58$	$27 \cdot 84 \pm 0 \cdot 74$
pp	$5 \cdot 36 \pm 0 \cdot 17$	$3 \cdot 08 \pm 0 \cdot 22$	$28 \cdot 94 \pm 0 \cdot 48$	$23 \cdot 41 \pm 0 \cdot 63$
Pp	$4 \cdot 53 \pm 0 \cdot 12$	$2 \cdot 80 \pm 0 \cdot 15$	$28 \cdot 74$	$27 \cdot 10$
$Ppcc$	$5 \cdot 01 \pm 0 \cdot 28$	$2 \cdot 70 \pm 0 \cdot 24$	$32 \cdot 37$	$25 \cdot 90$

the probable error in the definition of y is the least, in other words *as indicator of the value of the crossing-over we take the constant term of the equation of the straight line expressing the law of alteration of the size of the crossing-over by days of hatching.*

In the calculation of the standard error we calculated according to the corresponding equation the theoretical quantity for each day. Then we took the difference between the theoretical quantity and the observed empirical one, and from these deviations, considering of course their weight, we calculated σ, and from this the standard error.

But it is quite impossible to define directly the size of crossing-over between black and cinnabar for the $c_n c_n$ flies. We found the equation for the size of crossing-over between black and purple from Tables VII and VIII.

These equations are:

$$\text{for } A \text{ days } y = -0 \cdot 12x + 4 \cdot 09;$$
$$\text{for } B \text{ days } y = -0 \cdot 02x + 1 \cdot 95.$$

Then from Tables V and VI, which are much larger, we found the amount of crossing-over between purple and cinnabar.

The equations obtained are:

$$\text{for } A \text{ days } y = -0 \cdot 042x + 0 \cdot 92;$$
$$\text{for } B \text{ days } y = -0 \cdot 022x + 0 \cdot 75.$$

The amount of crossing-over between black and cinnabar can be found approximately by the summation of constant terms. This sum shows what would have been the size of crossing-over between black and cinnabar if it had been possible to define it in the $c_n c_n$ compound. These quantities (5·01 and 2·70) are shown in Tables IX and X.

The Correction of the General Size of the Crossing-over.

When we organised our experiment we introduced in the line of analysators the gene "dumpy" which was to serve a "controlling" function. As a considerable time was needed for the carrying out of our experiment, there might have occurred some changes, observed or unobserved, in the conditions of breeding of the culture. Therefore it was important to have some indication whether such changes had or had not occurred. The crossing-over on the long dumpy-black region was to be such a controlling indicator.

But it might also have happened that changes in the genotype, such as the substitution of the normal allelomorph for the recessive purple one, had completely altered the aptitude for crossing-over, affecting the size of flies in the same way as "the day of emergence."

On Table IX the permanent terms of the equation ($y_1 = \dots$) represent the size of crossing-over on the region T^d–b for the female of the 0-day of emergence. We can see that on the dumpy-black region flies of different structure gave different amounts of crossing-over, and it is evident that these differences are not due to chance, *i.e.* they are beyond limits of probable errors. So in the main line A, with the normal allelomorph (P) in the middle, the amount of crossing-over is 29·0 while in the line with pp substituted for PP it falls to 26·25.

We can observe the same difference in the black-cinnabar region, and therefore the question naturally arises: whether the PP cultures are not in slightly different conditions to the pp cultures, leading to a general diminution in the amount of crossing-over in the latter.

It is necessary to discuss this question carefully. First of all we must say that the two cultures showing this difference in the amount of crossing-over in the dumpy-black region, (PP and pp cultures) were in as nearly identical conditions as we could create in our laboratory, the jars containing them being interspersed, and the broods contemporaneous. Therefore we can reject the possibility of a difference in the external conditions. There remains the possibility that the diminution of size of the crossing-over on the dumpy-black region reflects the general diminution of size of the crossing-over in the purple flies.

But this is equally improbable since the comparison of different types of crossings does not reveal any parallelism between the size of crossing-over in the dumpy-black region and that in the black-cinnabar region. In Table IX one of the lowest values for black-cinnabar crossing-over corresponds to the highest value for dumpy-black crossing-over (32·37), viz. 5 to 5·7 per cent. for pp crossing and 7·2 for PP crossing. In the pp line 26·25 per cent., one of the highest percentages for the black-cinnabar region, is associated with the lowest percentage for dumpy-black crossing-over. On the table of B-days in the first or second region we observe the same absence of any connection between the amounts of crossing-over. In other words we may conclude that the differences in the amounts of crossing-over in the black-dumpy and black-cinnabar regions, which we observed in transition from the crossings PP to P and from pp to Pp, are local changes, peculiar to this region of the chromosome. We cannot say if this change is accompanied by other changes further to the left, but an examination of the material gives no ground for supposing that such changes exist.

We may now turn to the changes in the amount of crossing-over in the black-cinnabar region (see Tables IX and X).

(1) We can observe in all our experiments that the females of type PP, in both groups A and B, have the highest percentage of crossing-over between black and cinnabar. For the A group the quantity is 7·2 per cent. and for the group B 3·7 per cent.

(2) The substitution of PP by pp is accompanied by a reduction of the crossing-over to 5·4 for group A, and to 3·1 per cent. for group B. It seems as if a kind of shortening of the chromosome between black and cinnabar takes place here.

(3) The substitution of the homozygous type by the heterozygous type (Pp) reduces the amount of crossing-over to the level of 4·5 per cent. for A, and 2·8 per cent. for B.

(4) The substitution of C by c_n in the structure $\dfrac{T^d\ b\ p_r\ c_n}{}$ does not noticeably affect the crossing-over between black and cinnabar. We may present this result more clearly by transferring it to the black-purple region. In the structure $\dfrac{T^d\ b\ p_r\ c_n}{}$ the size of the crossing-over in this region is 3·61 (4·53–0·92) for A days, and 2·05 (2·80–0·75) for B days. The corresponding quantities for the structure $\dfrac{T^d\ b\ p_r\ c_n}{c_n}$ are 4·09 and 1·95. Having regard to the conditions of our experiment the differences between those quantities cannot be considered as significant.

We may turn now to Table X, in which we have summed up our results for flies of different constitution. Generally speaking such a summary is recommended. With the act of segregation in different structures arise different classes of crossing-over with different fitness for life, and this difference can alter a little the general total. To avoid this source of error, one ought to investigate the crossing-over first as a "coupling" and then as a "repulsion," and then deduce its value from these two separate values. An examination of Table IX shows us that sometimes there is a noticeable difference. For instance, in type $\dfrac{T^d\ b\ p_r\ c_n}{p_r}$ (*A*) the value of the crossing-over on the black-cinnabar region is $5 \cdot 68 \pm 0 \cdot 22$ and in type $\dfrac{T^d\ b\ p_r}{p_r\ c_n}$ the same crossing-over is diminished to $4 \cdot 34$. The difference in the amount of crossing-over in this region is especially noticeable in Tables V and VI on comparing 1 with 2, *i.e.* on comparing the results from $\dfrac{T^d\ b\ p_r\ c_n}{}$ flies with those from $\dfrac{p_r\ c_n}{T^d\ b}$ flies.

How is this difference called forth? For on the theory of the gene the amount of crossing-over in both coupling and repulsion should be equal. It would be very important to verify by special experiments how far this presumed equality extends. If a disturbance in the architectural symmetry of the chromosome has any influence on the value of the crossing-over then the structures discussed above, which are asymmetrical in different degrees, should give rise to different crossing-over values, the value being larger according as the symmetry is more perfect.

Therefore, although we have constructed Table X, we prefer to compare the results of the investigation of the most similar structures belonging to different compounds rather than summarise those relating to the different structures (Tables I–IX).

Thus the most similar structures in different compounds would be:

$$
\begin{array}{ccc}
PP \text{ compound} & pp \text{ compound} & Pp \text{ compound} \\
\dfrac{T^d\ b\quad c_n}{}\ \text{ and } & \dfrac{T^d\ b\ p_r\ c_n}{p_r}\ \text{ and } & \dfrac{T^d\ b\ p_r\ c_n}{} \\[2ex]
\dfrac{T^d\ b}{c_n}\ \text{ and } & \dfrac{T^d\ b\ p_r}{p_r\ c_n}\ \text{ and so on.} &
\end{array}
$$

Comparing compounds *PP* and *pp* we found that the results are in general alike in all structures. But with the *Pp* compound we do not find such uniformity. For flies of constitution 2 in Tables III and V, IV and

VI the crossing-over with Pp compound is lower than that with pp compound, whereas in the other Tables this relation seems to be reversed. Therefore we must acknowledge that any difference between Pp compound and pp compound remains unproved.

THE SIZE OF THE GENE P.

What is to be said of the size of the normal allelomorph "purple" in the light of these experiments? Since females of different days of emergence show different values for crossing-over, and since these values differ also in A and B cultures, and at different temperatures, we can speak of the length of this gene only conditionally. If we use the data for the A-days, the size of the P gene works out at $1\cdot85 \pm 0\cdot34$, a rather large size. However, in this value the statistically significant part will be only $1\cdot85 - (0\cdot34 \times 3) = 0\cdot83$ morg. For B-days the difference between PP and pp will be equal to $0\cdot60 \pm 0\cdot31$ morg. But the total value of crossing-over in B cultures as compared with A is lowered $1\cdot86$ times; therefore, if we transfer it to the scale of A-days, we shall have $1\cdot86 (0\cdot60 \pm 0\cdot31) = 1\cdot12 \pm 0\cdot58$. Averaging both determinations, we shall have $P = 1\cdot48 \pm 0\cdot33$, $i.e.$ again a rather large size, in which, however, the statistically significant part is only $1\cdot48 - (0\cdot33 \times 3)$, that is to say only $0\cdot49$ morg., which we can approximately take as the result of our determination of the size of the purple-gene. Here we ought to keep in view that according to the character of the method used, we are able to determine the significant difference between PP and pp, especially if we occasionally happen to determine a size greater than the real one; but if we happen to determine a size less than the real one, it disappears in the limits of the error in the experiment.

THE PRESENCE AND ABSENCE THEORY.

It is not difficult to see that the results obtained in the present investigation can be used for the resurrection of Bateson and Punnett's Presence and Absence theory, which seemed buried. As an epitaph to this theory we can quote the words of Morgan in the last chapter of his book on "The Physical Basis of Heredity," consecrated to the multiple allelomorph, that "only one kind of absence is thinkable" (p. 251).

However, our opinion is that the Presence and Absence theory can be brought into agreement with the Chromosome Theory of Heredity. Only both theories must give up the postulate of the indivisibility of the gene. If the results obtained in the present investigation are confirmed by investigations on other genes, it will perhaps be possible to

explain the series of allelomorphs by the hypothesis that in different mutations parts of chromosomes unequal in point of size disappear. If "white" is the result of the disappearance of a chromosomal region of the length of 0·10 morg., then "ivory" may be the result of the disappearance of a region of 0·05 of a morg., "eosin" of 0·03 morg., and so on. Each of these mutations will, according to Bateson, represent a real "absence," and will not at the same time belie Morgan's theory of chromosomes. Only Morgan would perhaps be obliged to change his nomenclature somewhat, because from this point of view "the normal allelomorph" for "eosin" will no longer be the same element as for "white." In the language of Morgan's symbols, we shall be compelled to speak of W and w, W^e and w^e, W^i and w^i, and so on.

This point of view is of interest in attributing a similar physiological influence to *whole areas* (even to those, having a length of several fractions of morg.) of chromosomes, contrary to the current view of the chromosome, as of a thread on which are strung various beads in a random order: one of the beads influencing the colour of eyes, the next the shape of the wing, and so on.

We consider it opportune to mention it here, because the question of the different length of the different members of a series of allelomorphs might be capable of experimental proof through the further development of the method we have used in the present investigation.

PART II. THE VARIATION OF RARE CROSSING-OVERS.

For the purpose of the present investigation it was very important to determine the sizes and sources of the variation of the crossing-overs under consideration, in order to decide whether this variation might not hinder the solution of our problem. The biometrical analysis, carried out by Gowen (1919), showed that the phenomenon of crossing-over is one that exhibits unusually great variation. This is markedly the case for the rarer crossing-overs, *i.e.* those between closely located genes; and even more so for double and triple crossing-overs. Estimates of this variation by means of the coefficient of variation give coefficients of exceptional value, reaching 100 per cent., and higher. This exceeds anything known about the variation of biological quantities.

In the centre of our investigation we placed the crossing-over between "black" and "cinnabar," as having the rather low value of about 5 per cent., and we may now enquire whether this value ever shows the extraordinary large variation referred to above. If such proved to be the case, we could hardly hope to establish the small change in the value

of the crossing-over which we suggest as arising through the substitution of one allelomorph P for another p.

From the analysis of data, given by Gowen in his Tables A–D we noticed that the coefficient of variation depended upon two causes: (1) upon the variation of the biological phenomenon itself, and (2) upon conditions of a purely methodological character, having no relation to the biological occurrence.

Let us take an example. The quadruple crossing-over, given by Gowen in Table D, represented such a rare phenomenon in his experiments, that among 31,456 flies, it was found in one sample only. Its occurrence is therefore 0·003 per cent. But in the bottle, where it was found there emerged 241 flies. Hence for this bottle, the percentage of quadruple crossing-overs was 0·416 per cent., that is to say 139 times more than the average value 0·003.

In this way for the given experiment we get:

$$\%$$

	%
In 254 bottles	0·000
In 1 bottle	0·416
Thence	$M = 0·00163$
	$\sigma = 0·0260$
	$C^{\%} = 1590.$

The coefficient of variation thus reaches the exceptionally high value of 1590 per cent. But are we justified in drawing such a conclusion as to the extraordinary variability of a quadruple crossing-over? Of course not. Since fractional parts of flies cannot emerge, this coefficient of variation could not be less than 1590 per cent. It would remain the same, so long as the quantity of flies in each single bottle were the same. But if ten times as many flies emerged from each bottle, a single fly, emerging in the bottle we were examining, would represent not 0·416 per cent., but only 0·0416 per cent.; and the coefficient of variation would have been markedly reduced, although the biological fraction of variation would remain unchanged.

It is obvious that the phenomenon of high variability is not due solely to the rarity or frequency of the crossing-over itself, but depends upon the appearance in a given bottle of a fly with a rare crossing-over, which is a concern of probability, not of biology. Evidently, the crossing-over value might remain the same, whether we investigate 10, 100 or 1000 flies in the same bottle. On the other hand, fluctuation on the base of random sampling would exhibit marked variability under these conditions.

To verify this we must determine whether the variability of rare crossing-overs depends upon the number of the flies emerging in the bottle. For this purpose we may use of our most homogeneous and extensive material, namely the cross

$$\varphi \; \frac{T^d \; b \; p_r \; c_n}{T^d \; b \; p_r \; c_n} \times \frac{T^d \; b \; p_r \; c_n}{T^d \; b \; p_r \; c_n} \; \male.$$

Firstly, let us see whether Gowen's statement holds good, that the rarer the crossing-over, the more marked is its variability. That it is so is evident from Table XI.

TABLE XI.

Types of cross-overs	Value of cross-overs $M\%$	Coefficient of variation $C\%$
Non-cross-overs	68·44	9·2
Single cross-overs:		
$T^d - b$	26·24	20·6
$b - p_r$	2·80	70·0
$p_r - c_n$	0·83	98·8
Double cross-overs:		
$T^d - b, \; b - p_r$	0·46	110·9

Now let us divide all bottles into groups as follows:

I group:	bottles with a number of flies less than 50			
II group:	,,	,,	,,	from 50 to 69
III group:	,,	,,	,,	from 70 to 89
IV group:	,,	,,	,,	from 90 to 109
V group:	,,	,,	,,	from 110 to 129
VI group:	,,	,,	,,	from 130 to 149
VII group:	,,	,,	,,	from 150 and more.

In Table XII are given the values of the coefficients of variation for each group of bottles separately.

TABLE XII.

The value of coefficients of variation for different groups of bottles.

Types of cross-overs	I	II	III	IV	V	VI	VII
Non-cross-overs	11·53	12·2	9·5	9·8	7·2	7·65	7·3
$T^d - b$	25·2	22·4	20·4	18·2	16·2	20·1	17·8
$b - p_r$	76·5	80·5	93·0	57·0	78·0	46·0	63·0
$p_r - c_n$	119·0	82·0	93·5	89·2	82·4	82·4	64·7
$T^d - b, \; b - p_r$	161	100	79	86	77	86	69

Generally speaking throughout this table we can observe the expected reduction in the value of the crossing-over percentage as we pass from group I to group VII. An irregular variation is to be observed only for the b-p crossing-overs. The rarer the crossing-over, the more marked the reduction in the percentage. Exceptionally high values occur in group I,

but in group VII they are already considerably reduced, and approach the usual biological coefficients of variation; and if we had groups of even larger size, *e.g.* VIII, IX, X and so on, it is fair to conclude that this reduction would continue.

Now arises the question whether the variability, on such further reduction, would yet retain a value which we should have to regard as unusually high. Or would such variability prove fairly normal? And could we therefore state that rare cross-overs should be more variable than more frequent cross-overs; double ones being more variable than single ones, triple ones more variable than double ones, and so on?

It is difficult to answer these questions from Table XII alone. It is necessary to find a method by which it would be possible to separate the biological part of variation from the mathematical one.

Let us denote by σ the standard deviation of variation of the crossing-over. This variation would depend on two elements: biological and mathematical. Let us denote the standard deviation of the biological part by σ_b and the standard deviation of the mathematical part by σ_m. Since these two variations are not correlated it follows that

$$\sigma^2 = \sigma_b{}^2 + \sigma_m{}^2.$$

From this we can determine σ_b, the biological part of the variation in which we are interested:

$$\sigma_b = \sqrt{\sigma^2 - \sigma_m{}^2}.$$

The size σ can be determined by experiment. The value σ_m can be calculated. If the probability of finding the given cross-over is denoted by p, and the probability of not finding it by $q = 1 - p$, then to determine σ_m we only need to know the number of flies emerged in the bottle. In the simplest case, when there will have emerged N flies in every bottle

$$\sigma_m{}^2 = \frac{pq}{N}.$$

But for us it is more convenient to have to deal not with p and q, but with percentage of crossing-over and with percentage of non-crossing-over, because these are also determined in the same quantities. Then

$$\sigma_m{}^2 = \frac{pq}{N} \cdot 100^2.$$

The calculation is in our case complicated by the condition that the number of flies emerged differs for different bottles. This is why, first of all, we are compelled to determine analytically to what extent σ_m may depend on the variation of the number of flies in the bottle.

Suppose we have a row of bottles with a number of flies

$$n, n_1, n_2, \ldots\ldots n_k,$$

in which are hatched flies of two categories, A and B, and here the *a priori* probability of A flies is equal to P, and of B flies equal to Q, and here $P + Q = 1$.

The variation of the number of A and B flies in the bottle with n_1 flies, is submitted to the binomial $(P + Q)^{n_1}$, and the standard deviation will be $\sigma_1 = \sqrt{PQn_1}$; thus, for different bottles we shall have a row of equations

$$\sigma_1 = \sqrt{PQ\, n_1}$$
$$\sigma_2 = \sqrt{PQ\, n_2}$$
$$\sigma_3 = \sqrt{PQ\, n_3}$$
$$\ldots\ldots\ldots\ldots\ldots$$
$$\sigma_k = \sqrt{PQ\, n_k}.$$

After squaring, we obtain

$$\sigma_1{}^2 = PQ\, n_1$$
$$\sigma_2{}^2 = PQ\, n_2$$
$$\ldots\ldots\ldots\ldots$$
$$\sigma_k{}^2 = PQ\, n_k.$$

After summing, we get

$$\sigma_1{}^2 + \sigma_2{}^2 + \ldots + \sigma_k{}^2 = PQ\, (n_1 + n_2 + \ldots + n_k).$$

After dividing by k we obtain the value of $\sigma_m{}^2$

$$\sigma_m{}^2 = \frac{\sigma_1{}^2 + \sigma_2{}^2 + \ldots + \sigma_k{}^2}{k} = PQ\left(\frac{n_1 + n_2 + \ldots + n_k}{k}\right).$$

Here σ_m represents the mean standard deviation of single bottles, and k denotes the number of bottles. The expression in brackets is nothing else but the average of the numbers of flies in the bottle. Thus we have

$$\sigma_m{}^2 = PQM,$$

i.e. the standard deviation, the mean average for the groups of bottles with a variable quantity of flies = to the standard deviation of the bottle, containing the mean number of flies.

Making the calculation—as in our case—in percentage, we ought to find the quantity

$$\sigma_{m\%} = \frac{\sigma_m}{M}\, 100\,\%.$$

We get it, squaring this equation

$$\sigma^2{}_{m\%} = \frac{\sigma_m{}^2}{M^2} \, 100^2,$$

as

$$\sigma_m{}^2 = PQM,$$

therefore

$$\sigma^2{}_{m\%} = \frac{PQM \cdot 100^2}{M^2} = \frac{100P \cdot 100Q}{M}.$$

Denoting $100\,P$ by p per cent. and $100\,Q$ by q per cent., we have

$$\sigma^2{}_{m\%} = \frac{p\% \cdot q\%}{M},$$

and

$$\sigma_{m\%} = \sqrt{\frac{p\% \cdot q\%}{M}},$$

that is to say, a standard deviation of percentage of crossing-overs for the group of bottles with a different number of flies, is equal to the σ of a bottle with the sum of all flies.

Let us see how the coefficient of variation is changed, thanks to the correction by σ_m.

(a) The variation of crossing-over on the distance, "purple-cinnabar" in 64 bottles of group A_1.

The number of flies cross-overs	The number of bottles
0	23
1	21
2	12
3	6
4	2
	64

$$\sigma = \pm 1 \cdot 09 \text{ flies,}$$

$$M = \frac{6994}{64} = 109 \cdot 3,$$

$$\sigma_\% = \frac{1 \cdot 09}{109 \cdot 3} \cdot 100 = \pm 0 \cdot 997 \, \%.$$

The percentage of crossing-over for this group is equal to $p\% = 1 \cdot 02\,\%$. From this

$$C^\% = \frac{0 \cdot 997}{1 \cdot 02} \cdot 100 = 98 \, \%.$$

But

$$\sigma^2{}_{m\%} = \frac{1 \cdot 02 \times 98 \cdot 98}{109 \cdot 3} = 0 \cdot 905;$$

$$\sigma^2{}_{b\%} = 0 \cdot 993 - 0 \cdot 905 = 0 \cdot 088;$$

$$\sigma_{b\%} = 0 \cdot 297.$$

Therefore already

$$C_b\% = \frac{0\cdot297}{1\cdot02} \cdot 100 = 29\%,$$

i.e. less than one-third of that calculated by ordinary means.

(*b*) Variation of crossing-over on the distance "black-purple," of group *A*, of the same 64 bottles.

The number of crossing	The number of bottles
0	1
1	11
2	12
3	9
4	8
5	9
6	6
7	6
8	2
	64

$$\sigma = \pm\ 2\cdot08 \text{ flies},$$

$$\sigma_\% = \pm\ 1\cdot91\%, \quad \sigma^2_\% = 3\cdot64,$$

$$p\% = \frac{233}{6994} \cdot 100 = 3\cdot33\%,$$

$$C\% = \frac{1\cdot91}{3\cdot33} \cdot 100 = 57\cdot3\%.$$

However

$$\sigma^2_{m\%} = \frac{3\cdot33 \times 96\cdot67}{109\cdot3} = 2\cdot95\%.$$

Whence

$$\sigma^2_{b\%} = 3\cdot64 - 2\cdot95 = 0\cdot69, \quad \sigma_{b\%} = 0\cdot83,$$

$$C_b\% = \frac{0\cdot83}{3\cdot33} = 100 = 25\%.$$

In these two examples it is obvious what a marked effect is brought about by the introduction of the correction in question. Without it the coefficients of variation for the distances b–p_r are 57·3 per cent. and for p_r–c_n 98 per cent.: with it they become 25 per cent. and 29 per cent., *i.e.* firstly they are both considerably reduced, and secondly they become nearly equal.

Returning to Gowen's data (see his Table III, p. 213), we may calculate the true biological variation of the crossing-overs he has investigated. Limiting ourselves to some examples chosen at random, we get the results shown in Table XIII.

TABLE XIII.

Region	% of crossing-over	Without correction		With correction	
		σ	$C\%$	σ_b	$C_b\%$
$s_e - r_0$	50·858	9·654	18·623	8·53	16·8
$s_e - D'$	10·900	3·807	34·923	2·57	23·6
$D' - c_u$	2·845	1·680	58·956	7·55	26·6
$s_e - D',\ D' - s_s$	1·065	1·029	96·651	0·19	18·0
$s_s - e^s,\ e^s - r_0$	0·613	0·672	109·750	0·00	0·0

It is not difficult to see that here also, the correction markedly affects the result. It leads to the disappearance of the difference between the variation of rare and of common crossing-overs, and of the idea that an increase of variation of crossing-overs is associated with a decrease in their frequency. The most common crossing-over $s_e - r$ has $C\% = 16$, and a very rare double crossing-over $s_e - D'$ and $D' - s_s$ occurring but one-fiftieth as often, has $C\% = 18$. We have nearly the same variation, whereas without our correction the numbers are respectively 18 and 96.

Thus we come to the conclusion, that between the biological variation of rare and common crossing-overs and double ones, there is no essential difference. The C per cent. fluctuates, according to the region of chromosomes involved, from 15 to 30 per cent.—*i.e.* has a size also usual for the variation of other biological variables. The variability of rare crossing-overs has given the impression of being exceptionally great, because former authors have not distinguished the biological part of variation from the mathematical one.

DISTRIBUTION OF RARE CROSS-OVERS.

Before we lit upon the above explanation of the exceptionally high variation shown by rare crossing-overs, as described by Gowen, we had framed certain hypotheses, one of which we may perhaps discuss. Up to the present time the moment of crossing-over is supposed to occur at an early stage of the maturation division in which alone occurs that conjugation of chromosomes which, on Morgan's view, is indispensable for the production of crossing-over. At no other moment are the allelomorphic chromosomes located parallel to one another, so as to offer that mechanical interchange of areas between chromosomes essential to Morgan's scheme.

But an exceptionally marked variation of crossing-over might, perhaps, result were we to admit an exchange of genes at some division prior to the maturation division. Then, as the result of an exchange followed by multiplication of the cross-over cells, there might arise not

Examples of records of cultures, in which was observed the coincidence of two crossovers.

+, normal.   d, dumpy.   p, purple.   c, cinnabar.

3646 A_1 ♀ $\dfrac{d\,b\,p\,c}{d\,b\,p\,c}$ × ♂ $\dfrac{d\,b\,p\,c}{d\,b\,p\,c}$ 11 VII — 25

Total 101

3850 A_{11} ♀ $\dfrac{d\,b\,p\,c}{d\,b\,p\,c}$ × ♂ $\dfrac{d\,b\,p\,c}{d\,b\,p\,c}$ 16 VII — 30

Total 121

one, but many eggs of the given type, and this might possibly lead to an interesting reconciliation between the chromosome theory of Morgan and Bateson and Punnett's reduplication theory. But the most important point would, of course, lie in the fact that we should be compelled to admit the possibility of crossing-over independently of the conjugation of the chromosomes, which would undoubtedly change the whole theory of crossing-over.

As the present work is based on Morgan's scheme of crossing-over, it is of great importance for us to examine the possibility of such an explanation of a high variation of crossing-over. We are faced with the question whether the cross-overs may not be distributed in "packets," and the best means of solving it is to study the distribution of rare cross-overs.

Actually during our experiments, we noticed many cases of remarkable coincidences in the appearance of rare cross-overs. As already mentioned, the number of flies emerging was noted every day and for every bottle. And it often happened that on the same day and in the same bottle there was a simultaneous emergence of two rare cross-overs. Some of these cases of coincidence are represented in Table XIV. The most startling case was the emergence of two triple cross-overs on the same day in the same bottle. Triple cross-overs for the genes dumpy-black-purple-cinnabar represent a very great rarity, and among 55,000 flies, they occurred only six times. Hence the simultaneous emergence of two examples in the same bottle on the same day is exceedingly unlikely.

It is true that in such exceptional cases there is always the possibility of error or contamination. Sources of error may be (1) the creeping into the bottles of unrelated flies, (2) the creeping into the bottle of an accidental female to lay eggs in it, (3) the contamination of the flies examined by foreign ones occasionally left in the ether bottle (where flies are narcotised), and (4) a wrong description of flies owing to an indistinct development of characters.

The case of the simultaneous occurrence of two triple cross-overs was discussed on the spot and no reason was found for suspecting an error. The size and the freshness of the flies proved that they had just emerged like other flies in the same bottle. All other records for the bottle contained nothing suspicious, and contamination by unrelated eggs was out of the question. Flies, eventually sticking to the sides of the ether bottle are always dead, and so on.

Nevertheless, isolated cases of coincidence, however remarkable,

are explicable on grounds of error or chance, and are not therefore of great interest. Evidently it is necessary to study how far the distribution of rare cross-overs agrees with the probabilities as calculated on the base of Morgan's theory.

EXPERIMENTS ON THE DISTRIBUTION OF RARE CROSS-OVERS.

Whether the distribution of rare cross-overs deviates from random sampling was investigated as follows. In the first place we tried to answer the following question: if on a given day in a given bottle there was observed a cross-over − 3 (between purple and cinnabar) what was the probability of finding another sample of this cross-over in the same bottle, (1) on the same day, (2) on the following day, (3) in three days, and so on—or conversely, a day earlier, two days earlier, and so on?

For this purpose we investigated all groups of bottles separately, beginning with the A group. In this group of cultures were chosen all cases of − 3 cross-overs. In all there were 82 such flies. Each of these 82 flies we reckoned by turn as emerging on a zero day, and according to it, we orientated all other flies emerging in the same bottle. For instance, in bottle N 3435 (see Table XIV) one − 3 cross-over there emerged on the 10th day. Taking this day as the zero day we reckoned the 11th day as the + 1st, the 12th as the + 2nd and so on, the 9th day as the − 1st, the 8th as the − 2nd, and so on.

Therefore we consider that on the + 1st day emerged the cross-over − 3, because on the 11th day in the same bottle there actually emerged the second example of this rare cross-over. At the same time we noted how many flies had emerged on all these days in the given bottle, because we needed these data for calculating the probability of the finding of the cross-over on a given day.

Then as our basis of reckoning we took the example of the cross-over − 3, which had emerged on the 11th day. Now the 10th day is equal to the − 1st and we mark: "on the − 1st day has emerged the cross-over − 3."

In the same way we dealt with cases of the emergence of other rare cross-overs, e.g. − 2 cross-overs (i.e. of flies with cross-overs on the second distance, between b and p_r) in relation to the emergence of − 3 cross-overs.

Thus we get three different tables:

A, on which the − 3 cross-overs are distributed on conditional days, in relation to the emerging of cross-overs − 3: B, on which the cross-overs − 2 are distributed on conditional days in relation to the cross-

TABLE XV.

Example of using the data of cultures NN 3435, 3646, 3767 and 3850
for the investigation of the distribution of rare cross-overs.

(a) Example of constructing table "*A*": distribution of cross-overs-3 on conditional days in relation to cross-overs-3.

Conditional days

NN of bottles	−14	−13	−12	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	+13	+14
3435																1													
3435														1															
3646																													
3767														1		1													
3767														1		1													
3767															2														
3850																				2									
3850										1					1														
3850										1					1														
Total										2				3	4	3				2									

(b) Example of constructing table "*B*": distribution of cross-overs-2 on conditional days in relation to cross-overs-3.

Conditional days

NN of bottles	−14	−13	−12	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	+13	+14
3435									2													2							
3436										2													2						
3646													1	2	1							2							
3767													1		2														
3767													1		2														
3767													1		2														
3850																1			1										
3850														1			1												
3850														1			1												
Total									2	2		3	2	3	8	1	1			1		4	2						

(c) Example of constructing table "*C*": distribution of the total number of flies on conditional days in relation to cross-overs-3.

```
 NN     -14 -13 -12 -11 -10  -9  -8  -7  -6  -5  -4  -3  -2  -1   0  +1  +2  +3  +4  +5  +6  +7  +8  +9 +10 +11 +12 +13 +14
3435                            3  10   6   5   8  10  12   3  12  11   6   6   3   4  13
3435                        3  10   6   5   8  10  12   3  12  11   6   6   3   4  13
3646                                                    9  13   9   0   6   1   8  12  10   6  15   7   0   5   0
3767                                               33  16  13  12   3   5   4   8   6   3   4   7   5   2   1
3767                                           33  16  13  12   3   5   4   8   6   3   4   7   5   2   1
3767                                       33  16  13  12   3   5   4   8   6   3   4   7   5   2   1
3850                                           10  12   9  10  14   7  14   9   9   2   4   3   2  12   4
3850                                       10  12   9  10  14   7  14   9   9   2   4   3   2  12   4
3850                                       10  12   9  10  14   7  14   9   9   2   4   3   2  12   4
Total                       3  33  40  29  66 128 100 106  78  62  55  44  59  47  70  44  38  22   7  19   4
```

overs — 3: and C, on which the total number of flies is distributed on conditional days in relation to the cross-overs·— 3.

On Table XV is given an example of how these tables are drawn up on the basis of the data from cultures NN 3435, 3646, 3767 and 3850 whose records are given in Table XIV. Actually these two cultures are not included in the common table, belonging to different days of emerging of females A_1, A_{11}, A_{111}, while we have drawn up separate tables for females of every day and there were constructed whole ranges of tables: A_1, A_{11}, A_{111},...B_1, B_{11}, B_{111},...C_1, C_{11}, C_{111},....

The method of using these tables is as follows. From A and C we can determine the probability of the finding of — 3 cross-overs for each conditional day, and so know, whether it remains more or less constant, or is subject to definite variations. If the probability undergoes a change, it can be, first of all, explained as a sign of fluctuation of the crossing-over

TABLE XVI.

Distribution on conditional days in relation to cross-overs-3, findings of cross-overs-3 and the probability of their finding (p. 100).

Group of bottles: A_1.

Conditional days	Total number of flies (a)	Number of cross-overs-3 (b)	$\frac{b}{a} \cdot 100$
− 14	16	0	0·00
13	23	0	0·00
12	20	0	0·00
11	30	1	3·34
10	59	2	3·39
9	102	1	0·98
8	84	1	1·19
7 ·	111	1	0·90
6	165	6	3·64
5	271	6	2·21
4	301	4	1·33
3	302	3	1·32
2	335	4	1·19
− 1	438	3	0·68
0	682	18	2·64
+ 1	535	3	0·55
2	428	4	0·93
3	412	3	0·73
4	371	4	1·08
5	353	6	1·65
6	353	6	1·70
7	291	1	0·34
8	254	1	0·38
9	210	1	0·48
10	155	2	1·29
11	172	1	0·58
12	100	0	0·00
13	72	0	0·00
+ 14	37	0	0·00

itself. But then it is obvious that the same fluctuation ought to show the probability of the finding of cross-overs — 2. This probability can be calculated by using Tables B and C.

Let us now proceed to the examination of the actual data. The study of one of the largest groups of bottles A_1 (see Tables XVI and XVII) shows a very peculiar fluctuation in the probable appearances of — 3 cross-overs. It turns out that on the day when a — 3 cross-over is found the probability of the finding of a second example of a cross-over of the same type is very great. Thus for the zero day this probability is 2·64 per cent., *i.e.* five times more than the average probability (0·5 per cent.). (When speaking of probability, we shall have in view not p but $p.100$, for this is namely the size, measuring the distance between the genes.) But on the following day and on the day before, the probability decreases to its average size (0·56 and 0·68). After this again the farther from the

TABLE XVII.

Distribution on conditional days in relation to cross-overs-3, findings of cross-overs-2, and the probability of their findings ($p . 100$).

Group of bottles: A_1.

Conditional days	Total number of flies (a)	Number of cross-overs-2 (c)	$\frac{c}{a} . 100$
−14	16	1	6·25
13	23	0	0·00
12	20	1	5·00
11	30	2	6·57
10	59	1	1·69
9	102	4	3·92
8	84	2	2·39
7	111	4	3·60
6	165	8	4·85
5	271	4	1·47
4	301	8	2·65
3	302	8	2·65
2	335	22	6·57
−1	438	20	4·57
0	682	29	4·25
+1	536	20	3·73
2	428	25	5·84
3	412	15	3·65
4	371	19	5·12
5	363	8	2·20
6	353	13	3·58
7	291	12	4·12
8	264	11	4·16
9	210	9	4·28
10	155	4	2·58
11	172	5	2·90
12	100	1	1·00
13	72	2	2·78
+14	37	0	0·00

zero day, the higher the probability of finding another example, until on the $\pm$ 6th day it again reaches a high size (1·70–3·60). Then the probability decreases again until it shows a further rise about the $\pm$ 10th day. However, for establishing this third rise, our material is too scanty.

But what is the significance of such marked fluctuations? Why, on the day when a -3 cross-over is found, is there so great a probability of finding a second example of the same type?

In the first place it is possible that these days correspond to some general rise in the frequency of crossing-overs. Therefore we should expect the adjacent distance $b-p_r$ also to show on these days a greater frequency of cross-overs. Table XVII shows clearly that this expectation is not borne out. On the 0 day, as likewise on the adjacent days, the

TABLE XVIII.

Distribution on conditional days in relation to cross-overs-3, findings of cross-overs-3, cross-overs-2 and probability of their finding (p . 100 and p' . 100).

Group of bottles: A_{11}.

Conditional days	Total number of flies (a)	Number of cross-overs-3 (b)	Number of cross-overs-2 (c)	$\frac{b}{a}.100$	$\frac{c}{a}.100$
− 14	18	1	2	5·55	11·12
13	97	1	12	1·03	12·38
12	73	1	2	1·37	2·75
11	96	3	5	3·12	5·20
10	108	0	7	0·00	5·92
9	139	2	6	1·44	4·31
8	145	3	5	2·07	3·45
7	158	3	6	1·90	3·80
6	221	2	18	0·91	8·15
5	227	8	14	3·53	6·15
4	240	2	6	0·84	2·50
3	238	4	11	1·68	4·62
2	297	1	12	0·33	4·05
− 1	494	4	22	0·81	4·45
0	600	10	35	1·67	5·83
+ 1	442	4	17	0·91	3·85
2	378	1	13	0·26	3·44
3	360	4	13	1·11	3·62
4	318	2	13	0·63	4·09
5	287	8	5	2·79	1·74
6	247	2	4	0·81	1·62
7	190	3	6	1·58	3·15
8	222	3	4	1·35	1·80
9	163	2	7	1·23	4·29
10	178	0	1	0·00	0·56
11	126	3	0	2·38	0·00
12	93	1	4	1·07	4·30
13	59	1	0	1·69	0·00
+ 14	44	1	0	2·27	0·00

probability of finding cross-over − 2 remains almost unchanged and practically normal, being about 4–5 per cent.

Hence the sharp increase of crossing-overs on the distance $p_r - c_n$ on the 0 conditional day is not accompanied by any rise in the number of crossing-overs in the closely adjacent region $b - p_r$. We are led therefore to infer that on that particular day only a *very small* part of the chromosome $p_r - c_n$ was affected, and that there were no specially favourable conditions for crossing-overs in general.

We may now turn to the analysis of the second group of bottles A_{11}, the data of which are given in Table XVIII. The distribution of cross-over − 3 here also shows the same features, though less sharply expressed. On the 0 day the probability is again strongly raised, viz. to 1·67 per cent., but only twice as much as compared with the adjacent days (0·81 and 0·91 per cent.). Farther on, about ± 5 day there is a fresh rise. We cannot judge of further fluctuation because the data are too scanty.

Nevertheless, on the zero day, the probability in this group of the finding of cross-over − 2 also shows a slight rise. However, the fluctuations are here less significant:

cr. − 2	4·05	4·45	5·83	3·85	3·44
cr. − 3	0·33	0·81	1·67	0·91	0·26
or in %					
cr. − 2	69	76	100	66	59
cr. − 3	20	48	100	54	16

Obviously these figures for group A_{11} are to be interpreted as meaning that on the days when − 3 cross-overs were found, the probability of finding also the − 2 cross-overs was somewhat raised. But in a far higher degree had risen the probability of finding a second example of a cross-over of the same type, *i.e.* cross-over − 3.

All that was established for group A_{11} of bottles, proved right for all the remaining material. In the distribution of the rare − 3 cross-overs there appeared two peculiarities:

(1) On the day of finding cross-over − 3 there clearly appears a greater probability of finding a second example of the same type $(p_r - c_n)$; and the probability of finding cross-over − 2 $(b - p_r)$ also rises, though in a less degree.

(2) The probability of finding cross-over − 3 again rises on the 5th and 6th days, and perhaps also on the 10th–12th days. It was also established that on the 5th–6th days the total of flies emerging also rose a little, but, of course, the increased probability of finding cross-over

— 3 is not for this reason invalidated, because it is already calculated according to this increased number of flies emerging.

What then may we conclude from these facts? They show a somewhat peculiar periodicity of cross-overs, or at any rate in the emergence of cross-over flies. This periodicity seems to coincide with the periodicity of egg-laying: during periods of more active egg-laying, the absolute number of cross-overs emerging not only increases, but their percentage is also somewhat higher. As might have been expected, from Gowen's data the crossing-overs on the adjacent distances $b - p_r$ and $p_r - c_n$ show some slight positive correlation; and this is to be explained by the circumstance that the females included in a group, as for instance A_1, can nevertheless differ in age by as much as 24 hours, and that is why their crossing-over capacity undergoes some variations.

It is, however, probable that at certain times in the region $p_r - c_n$ investigated, notwithstanding its small size, there occurs a considerable increase in the probability of crossing-over. This increase is of such a kind, that at such times there are formed in the female from two to five eggs with a crossing-over between p_r and c_n.

How is such a phenomenon to be interpreted? For on Morgan's view, crossing-over at this or that point occurs at random, and with this the facts just related do not agree.

If we insist that the crossing-over occurs during a chromosomal conjugation and that it results in the formation of only one egg of the given type, we must admit that the position of the crossing-over proves to be not quite at random, but even rather regular. If on a given day in one cell, crossing-over has occurred at a point between p_r and c_n, then we must suppose that at the same time in another cell also some force is tending strongly to produce crossing-over at this same point between p_r and c_n. To regard this force as temperature, light, dampness or some other external agent is very difficult, and we may be compelled to look for it inside the cell itself, perhaps as some form of chemical force.

If, however, we insist on the random character of crossing-overs at the given point, their originating in pairs or in more numerous groups, might be explained on the hypothesis that crossing-over occurs, not only at the maturation division, but at earlier divisions also.

The present work is, however, not designed for resolving so important a problem, for which indeed special experiments are needed. It is sufficient here to point out the observed deviations, and to emphasise that they are not frequent enough to hinder the definition of crossing-over with the exactness we need for the purpose of the present investigation.

SUMMARY.

1. A theoretical discussion on the question of the nature of the gene suggests that the gene is a part of the chromosome. From this suggestion arises the problem of measuring the length of the gene.

2. In the present work an attempt has been made to measure the gene "purple" (p_r) by the method of comparing the value of crossing-over between "black" (b) and "cinnabar" (c_n) in the following crosses:

$$(1) \quad \frac{}{b \quad c_n} \times \frac{b \; p_r \; c_n}{b \; p_r \; c_n};$$

$$(2) \quad \frac{}{b \; p_r \; c_n} \times \frac{b \; p_r \; c_n}{b \; p_r \; c_n};$$

$$(3) \quad \frac{p_r}{b \; p_r \; c_n} \times \frac{b \; p_r \; c_n}{b \; p_r \; c_n};$$

and in some other instances.

3. The value of the crossing-over between b and c_n turned out to be the greatest in (1), *i.e.* when between b and c_n were the normal allelomorphs for p_r, *i.e.* P. The substitution of PP for pp diminished the crossing-over, that is to say, "shortened" the chromosome between b and c_n.

4. In the case of asymmetrical construction of the homologous chromosomes, as in (2), that is to say with Pp, there possibly occurred the most marked decrease of the crossing-over. However, the difference between Pp and pp turned out not to be certain.

5. There was discovered a source of marked variation of crossing-over. Flies of a brood which emerge earliest show the highest crossing-over values. Flies emerging on the following days show lower and lower crossing-over values. For the first ten days of emergence the value of the crossing-over is a linear function of the day of emergence.

6. The variation of a crossing-over does not represent something exceptional, as was stated by Gowen (1919). The high coefficients of variation obtained by him ($C\% = 100$ and more) are not entirely biological in nature, and depend upon the method of calculation.

7. However, in the distribution of rare cross-overs there exist somewhat peculiar deviations from expectation on random sampling. It is possible that crossing-overs of the same type may arise by groups, and a decision with regard to this possibility would be of great importance for the theory of crossing-over.

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THE ATTACHMENTS OF CHROMOSOMES AT THE REDUCTION DIVISION IN FLOWERING PLANTS.

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(With Sixteen Text-figures and Six Diagrams.)

CONTENTS.

INTRODUCTION.

It is usually difficult to discover how the homologous chromosomes (homologues) are attached at the thin-thread stages of the maturation divisions, or indeed at any stage before the late prophase, in flowering plants. However, in iron-acetocarmine preparations, where fixation in pollen-mother-cells is rapid and perfect (and also in smear preparations fixed with the best fixatives), the connections of the homologous chromosomes are usually visible, more or less clearly, at the late prophase and the first metaphase. Over 30 species and many varieties of flowering plants have been studied by the writer with regard to this point. The most important were the chromosomal varieties of *Datura Stramonium*, many varieties and species of *Canna*, five varieties of *Hyacinthus orientalis*, and two species of *Uvularia*. We will here neglect whatever happens in the pollen-mother-cells before the late prophase (diakinesis stage of many writers), and consider only the chromosomal connections found there, and at the following reduction metaphase. These connections are important; since, however they originated, they determine the manner of separation of the homologues at the reduction division. (It is proved

by the assortment in haploids and triploids that the reduction division is the first of the two maturation divisions.)

The whole number of the chromosomes in an undivided cell or nucleus is here called a "group." A "set" comprises homologous chromosomes only. "Diploid," "triploid" and "tetraploid" are used here only in their strictest sense, for plants having n sets of two, three, and four homologous chromosomes respectively; or for one such set alone.

UNIVALENTS OF HAPLOID PLANTS.

At the first metaphase of the haploid Datura, which cannot well be distinguished from the late prophase, the following points are noteworthy (Fig. 1). (1) The chromosomes are straight (Belling and Blakeslee, 1923), or nearly straight, and remain straight throughout the anaphase (Fig. 2). This indicates that the ordinary V-bend of the first anaphase
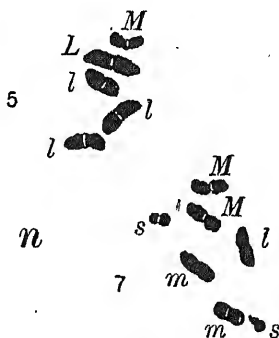

Fig. 1.   Fig. 2.

Fig. 1. First metaphase in pollen-mother-cell of haploid plant (Datura). The 12 chromosomes are not in contact nor attached to one another. They are straight or nearly so. The median constriction is visible, but no longitudinal split. Iron-acetocarmine preparation.

Fig. 2. First anaphase in pollen-mother-cell of haploid. The 12 chromosomes have proceeded at random to the poles, just as if each had its absent partner. The commonest division is 5 + 7. There is no longitudinal split yet visible. Iron-acetocarmine preparation.

chromosome in the diploid Datura is due to the pull of the spindle fibre at the centre having to break the connections between the two homologues which form a ring. (Also, in the diploid Datura, any two chromosomes attached only at one end, as happens in a small percentage of cases, are straight.) For the same reason, probably, these unpaired chromosomes of the haploid plant show no short tapering chromatin thread proceeding from the constriction along the spindle fibre as if

pulled out by this; as is seen in the bivalents, trivalents or quadrivalents of Daturas and most other plants. The unpaired chromosome (univalent) in $2n-1$ branches of Datura (Blakeslee and Belling, 1924) is also straight at the metaphase (Fig. 3); as is too the extra chromosome in the $2n+1$ primary mutants, when, as happens not rarely, it is separate from its bivalent at the metaphase (non-conjunction). The same is the case with any chromosomes of the triploid and tetraploid Daturas which show non-conjunction. But in plants with long chromosomes, such as *Uvularia*,
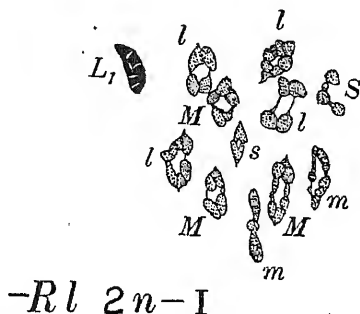

Fig. 3. First metaphase of a $2n-1$ pollen-mother-cell from a mutated branch of a diploid Datura plant. This branch showed 23 chromosomes in all the pollen-mother-cells of its flower buds. The unpaired chromosome is the largest, and is straight.

the long chromosomes are bent, even in cases of non-conjunction (Belling, 1925 b), apparently by the resistance of the cytoplasm as they are drawn to the poles by the spindle fibres. Hence we may perhaps deduce that the bend in the chromosomes at the anaphase of the reduction division (in chromosomes with median or subterminal constrictions) is a mechanical consequence of the pull of the spindle fibre, and the resistance to fracture of the two junctions with the homologous chromosome which is proceeding in the opposite direction; or, in long chromosomes, to the resistance opposed by the viscous cytoplasm to an object moving in it. The short chromosomes, VI and VII of Uvularia (Belling, 1925 b), which have a round segment connected with the body of the chromosome by a thread often as long as the chromosome, show this viscosity of the cytoplasm by the acute angle at the junction of the thread with the large segment at the anaphase in the pollen-grain.

(2) The twelve univalents of the haploid Datura show no attachment to one another (Belling and Blakeslee, 1923). This seems to prove that the 24 ends have different attractions; that is, that none of them are homologous. These haploid Daturas came from diploids by the parthenogenesis of an

egg-cell (Blakeslee, 1923). From a tetraploid, also, regular diploids have come by parthenogenesis (Blakeslee, 1923), having their chromosomes attached, as usual, in pairs (bivalents) at the reduction division. Hence the formation of a plant with half the number of chromosomes by partheno-genesis (induced in the diploid Datura by cold, or by attempted crosses with pollen of another species, which only occasionally succeed; and in the tetraploid by crossing it with pollen of diploids, which rarely produces a seed) would be a test in the case of the so-called tetraploid and hexa-ploid wheats, *Crepis biennis* (Collins and Mann, 1923) and similar plants; for if the half number of chromosomes at the reduction division had any homologous ends, these would combine (as in *Crepis biennis* in crosses) to form bivalents. In species crosses, some or all of the chromosomes in the reduction metaphase of the F_1 often show no attraction, and remain as univalents, while others may be mutually connected by one end only. Thus in the Cannas "Austria" and "Italia," which are doubtless F_1 plants of crosses of *Canna flaccida* of Florida, which has nine pairs of chromosomes, with another species of nine-chromosome Canna, only one to three pairs are formed (apparently united only at one end), and the rest of the 18 chromosomes are distributed seemingly at random.

We may perhaps postulate a gene causing the attraction at each end of each of the n different chromosomes of Datura and most other flower-ing plants. If this is so, it is probable, from what we know of gene mutation, that some or all of these genes might come to be different in allied species (or varieties), so that one or more chromosomes would not attract their homologues from the other species at one or both ends. If so, this would be a common cause for different degrees of sterility in the first generation hybrids of species crosses.

(3) The 12 chromosomes of the haploid Datura at the reduction meta-phase seem to pass at random to the two poles (Belling and Blakeslee, 1923), just as if their normal partners had been present. Miss Carothers has proved the random passage of the maternal and paternal homologues to the two poles at the reduction division in diploid grasshoppers (Carothers, 1921), where some homologues showed differences in the point of attach-ment of the spindle fibre. In the haploid Datura, the writer's observations show a distribution at the reduction division approximating to the terms of the binomial $(1 + 1)^{12}$, showing that the assortment is probably a random one. This seems to prove that the random assortment is not merely a conse-quence of the random orientation of the bivalents in the equatorial plane; since it takes place in the haploid group, which contains no bivalents. This random assortment in the haploid is hence probably a consequence

of the equality of attraction from the two poles. Also it has been shown that the extra chromosomes of each of the three different sizes in the pollen-grains of a triploid hyacinth went to the two poles of the pollen mother-cells at random (Belling, 1924). Hence the direction in which the spindle fibre pulls a chromosome is a random one; and this is probably explained by assuming that the fibre takes its origin from the chromosome. For the writer has several times found detached chromosomes outside the second metaphase plates in pollen-mother-cells of triploid and other Daturas, but, notwithstanding this position, divided into two, and with the halves pulled apart to a short distance by spindle fibres apparently not connected with any of the four poles of the second metaphases. Possibly a gene should be assumed for the origin of the spindle fibre, since its position is inherited (Carothers, 1921).

BIVALENTS OF DIPLOID PLANTS.

(1) The chromosomes at the reduction metaphase, in many diploids which have median constrictions, like Datura, form simple vertical rings (Diagram 2), in which the upper half of the ring with its two split halves (chromatids) goes to the upper pole, and the lower half to the lower pole. The connections become thinner and thinner until they break. As already stated, the homologous chromosomes are sometimes connected only at one end. Cases of non-conjunction also occasionally occur. (The origin of the V-shape of the chromosomes of bivalents at anaphase has already been considered.)

Fig. 3 A. Camera drawing of the reduction metaphase in *Rhoeo discolor*, showing all the univalents in a chain or split ring.

(2) In some diploids, as in certain Oenotheras, well investigated by Cleland, and also in *Rhoeo discolor* (Fig. 3 A), as the writer has found, the univalent chromosomes are sometimes united at the reduction

metaphase into a chain of **V**'s, alternate chromosomes passing to opposite poles. In Rhoeo, this chain is usually bent round to form a split ring; but in Oenothera there is stated to be a continuous ring (Cleland, 1924). In *Tradescantia virginiana*, the 24 univalents are, in the one plant examined, united more or less into rings of four; but the presence of a continuous chain is not excluded. Such junctions of non-homologous chromosomes of course postulate different attractions for one end of each of the two homologues, which attract certain non-homologous chromosomes at this end. Measurements are being made on Rhoeo to find if the sequence of chromosomes in the chain is invariable. Unless there is selective elimination of half of the progeny, only half of the offspring should show the continuous chain, the others having n bivalents at the reduction metaphase.

(3) In certain diploids, the shorter chromosomes are often, or perhaps in some pairs, always, attached at the points of constriction (points of spindle-fibre attachment) only; especially if these points are not median. This is the case usually with the shorter chromosomes (and sometimes with the medium and long chromosomes also) of *Hyacinthus orientalis*. It also occurs sometimes with the short chromosomes of Uvularia. In such **V**'s (or rather, truncated **A**'s) the two long segments may sometimes separate so as to send one chromatid to each pole, the whole forming an oval loop at the early anaphase (Belling, 1926).

(4) In the Drosophilas, and the few other organisms which have been investigated completely enough in this respect, it has been shown by several investigators, in one or more cases at least: (1) that crossing-over does not occur in a large fraction, perhaps somewhat less than a half, of the chromosomes; (2) that single crossing-over occurs frequently; (3) that double crossing-over is much less common; and (4) that triple crossing-over is rare, in any particular class of chromosome. In most flowering plants whose first-metaphase chromosomes have been examined by the writer, the members of the pairs (homologues) are connected, as already stated, only at the ends. They thus show no signs of any crossing-over (segmental interchange) which may have taken place previously. But the long chromosomes (though not the shorter ones) of Hyacinthus and Uvularia show, in addition to junctions at the ends, other junctions at which the chromatids appear under the microscope to be interlaced. These cases are given in Diagram 1, which is taken mainly from the four largest chromosome pairs of the hyacinth, though the largest bivalent of Uvularia is nearly similar. They show the following points: (1) In a majority of cases there is one definite point of fusion (node) where there is inter-

lacing of the chromatids as indicated by the microscope. (Diagram 1, a, b, c, d and e; Diagram 1 A, a.) (2) In somewhat fewer cases there are two such nodes. (Diagram 1, f and g; Digram 1 A, b.) (3) Cases of three nodes are rare. (Diagram 1, h.) There were found, in Hyacinthus, 62 bivalents with a single node, and 54 bivalents with two nodes, out of 116 examined. This would give, after completion of the first and second
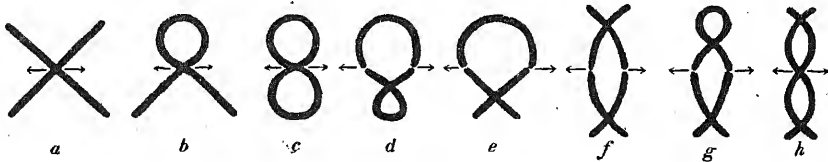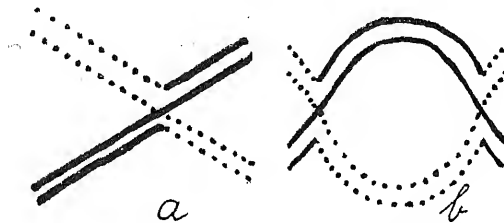

Diagram 1.

Diagram 1 A. Diagram of the structure of the nodes in the bivalents of *Hyacinthus orientalis*, as seen by changing the focus of the microscope.

divisions, 38 per cent. of chromosomes showing no segmental interchange, 50 per cent. with one point of interchange, and 12 per cent. with two points of segmental interchange. It seems an allowable working hypothesis that this parallelism with the genetic results in Drosophila indicates that such configurations in Hyacinthus and Uvularia may be consequences of crossing-over (segmental interchange).

THE TRIVALENTS OF TRIPLOID AND PRIMARY $2n + 1$ PLANTS.

(1) The trivalents of short chromosomes with median constrictions, such as those of Datura and Canna, either form some of five different configurations (Diagram 2, lowest line); or one homologue shows separation (non-conjunction) from the other two, which form a bivalent, usually a ring. These five are all the configurations which have been seen among several hundred trivalents of triploids (Fig. 4) and primary $2n + 1$ forms (Figs. 5 and 6) in Datura and Canna. Theoretically these are all the possible configurations for end-to-end union of homologues with different attractions at each end, and different attractions in each

Diagram 2.

$3n-1$

Fig. 4.

$2n+1$
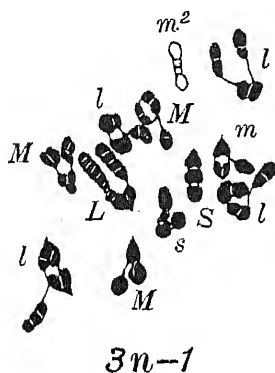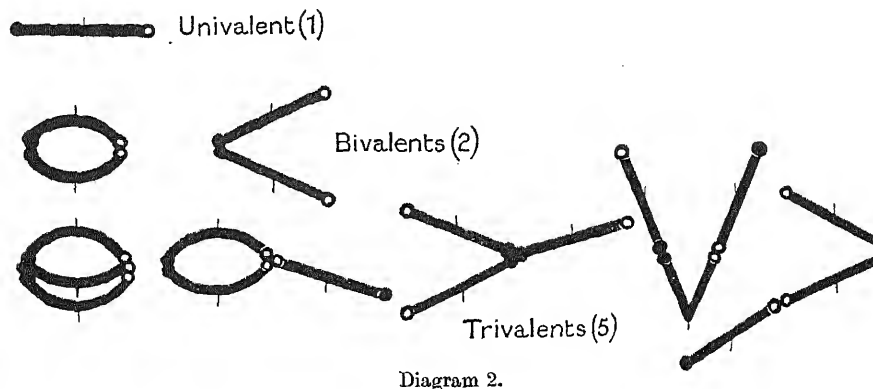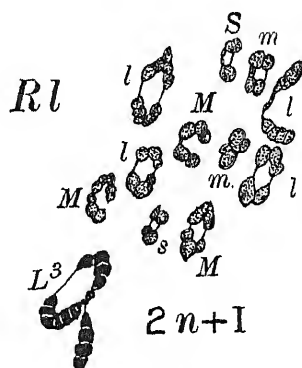
Fig. 5.

Fig. 4. First metaphase in a modified triploid, $3n-1$ plant. There are eleven trivalents, showing five open V's, six ring-and-rod configurations (including the doubtful S), and one bivalent, m^2. The chromosomes of any one trivalent are of the same size. Cytoplasm and chromosomes were removed from the cell-wall and flattened on the cover-glass. Iron-acetocarmine preparation.

Fig. 5. Camera drawing from an iron-acetocarmine preparation of the group of chromosomes at the first metaphase of the $2n+1$ primary mutant, Rl. The plant is triploid for chromosome 1, and the trivalent shows the ring-and-rod configuration. Eleven bivalents are also seen. Drawn from a preparation in which the cytoplasm and chromosomes had been squeezed from the cell-wall, and adhered to the coverglass.

of the n kinds of chromosomes. (There have been three exceptional trivalents found among those examined, but alternative explanations are probable for two of these, and possible for the other.) Of these five configurations, the V is the commonest, and it occurred in about half of the trivalents. This V could not form a ring of three by the two free

ends combining, for they are not homologous. The ring and rod is not far behind the V in numbers. Together they formed 70 per cent. of the trivalents. The three-rayed trivalent, or Y, is the next most numerous form. Some Y's seem horizontal at metaphase, but all are vertical when the anaphase commences. This configuration is often found in the smaller chromosomes of Datura instead of the ring and rod. The chain of three need not always be straight; but it differs from the V in the central chromosome not being bent, and in the two terminal chromosomes going

$2n + 1$ Rl ⌊_____⌋
                                        10μ

54.9   ±   0.9
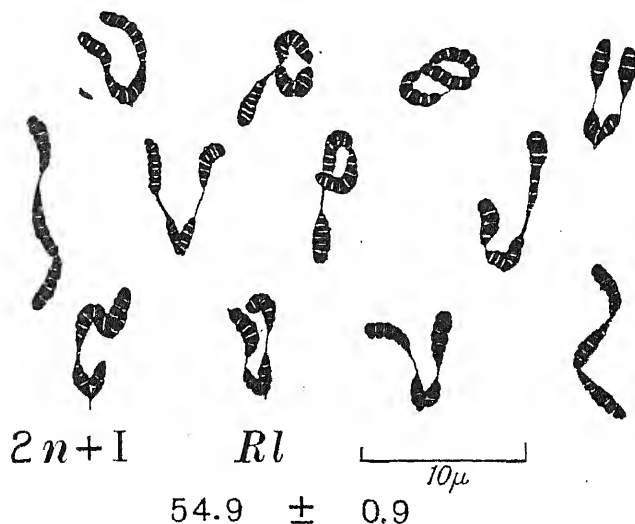
Fig. 6. Trivalents of the $2n+1$ primary mutant Rl, which is triploid for chromosome 1, the largest chromosome. The trivalents were at the first metaphase in the pollen-mother-cell. The drawings were made from iron-acetocarmine preparations. These trivalents were drawn with the camera, under a Zeiss apochromatic objective 60 of 1·3 aperture. As shown by the scale of 0·01 mm., the enlargement, at table level, was 2100. An oil-immersion objective was used, because the water-immersion would have showed changes of magnification for different coverglasses. The open V occurred in six cases, the ring and rod in three, the chain of three in two cases, and there was one example of the triple arc. This figure should be compared with Fig. 9, which depicts the trivalents of the corresponding secondary Sg; and also with Fig. 12, which shows chromosome 1 in the trivalents of Wy.

to different poles. The triple arc, in which all the ends of the three chromosomes are combined, might have been considered likely to be the most abundant configuration of the trivalents; but, in fact, it is the rarest. A single chromosome is, as already stated, found not uncommonly. It is always more or less straight, not bent into a small ring (as it is in secondary $2n + 1$ mutants).

If we count up the different attachments in the more or less random

sample of trivalents from ten of the primary $2n + 1$ (trisomic) forms given in the 1924 paper (Belling and Blakeslee, 1924 b), omitting one exceptional hexagon (which may perhaps have been a trivalent giving rise to a secondary, for it was open at one angle), we have, for 108 trivalents, 198 free ends, 147 junctions of two ends, and only 52 junctions of three ends. If the junction of three ends were as stable as that of two; or if two chromosomes having combined by the ends in the thin-thread stage, there still remained as much free attraction in this double end as there was in the single end; then there should be, in almost all trivalents, three ends combined (as two ends are nearly always combined in the diploid Daturas and Cannas). Therefore, the union of two ends hinders union with another homologous end. Hence, also, arises the frequency of the occurrence of separate single chromosomes (univalents) in the triploids, as compared with the diploids, where they are rare. In the true triploid Cannas, for instance, almost every other pollen-mother-cell may sometimes show one or two separate univalents.

On the whole, the absence of rings of three in the triploids of Daturas and Cannas, and in the trivalents of the 12 primary $2n + 1$ Daturas, indicates that the configurations originated by homologous ends of the chromosomes and no others coming together, as in diploids.

(2) Short chromosomes with subterminal constrictions, like the medium and short chromosomes of the triploid hyacinth, form more complicated configurations than are seen in the triploid Cannas and Daturas. Such homologous chromosomes may be connected either at the ends, or at the constrictions. In the latter case, as in the diploid hyacinth, the attraction may perhaps be supposed to reside in the chromosome ends on each side of the constriction. Some of the peculiar configurations resulting from this may be seen in Figs. 3 and 4 of the writer's paper on the chromosomes of the hyacinth (Belling, 1925 a). Some of the trivalents of *Hemerocallis fulva* resemble these.

(3) In the long chromosomes of the triploid hyacinth, which have a median constriction, the ring and rod and the chain of three have been demonstrated. But the three homologues may have connections at the constrictions, and also other connections like those already found in the long chromosomes of the diploid hyacinth. These other connections seem to be of the nature of interlacings of the chromatids, as in Uvularia (Belling, 1926). If such secondary connections ever occurred in the short chromosomes of the hyacinth, they have left no traces in the late prophase and metaphase; and this is also the case with all the chromosomes of Datura and Canna.

THE QUADRIVALENTS OF TETRAPLOIDS.

The tetraploids of *Datura stramonium* and of *Primula sinensis* were examined. In the Primula, the chromosomes were small, and squeezing out the contents of the pollen-mother-cell without injuring the quadrivalents was not successful. However, in some cells it seems that the homologues were combined in quadrivalents. In Datura, on the other hand, quadrivalents could be readily seen in all perfectly fixed cells, after squeezing out the cytoplasm and chromosomes. It was, however, difficult to get a preparation showing clearly all twelve quadrivalents uninjured. Figure 7 is a camera drawing of the best preparation obtained in the course of months. The configurations would of course be different for tetraploids with long chromosomes, and also with chromosomes showing subterminal constrictions. In the tetraploid Datura, all the
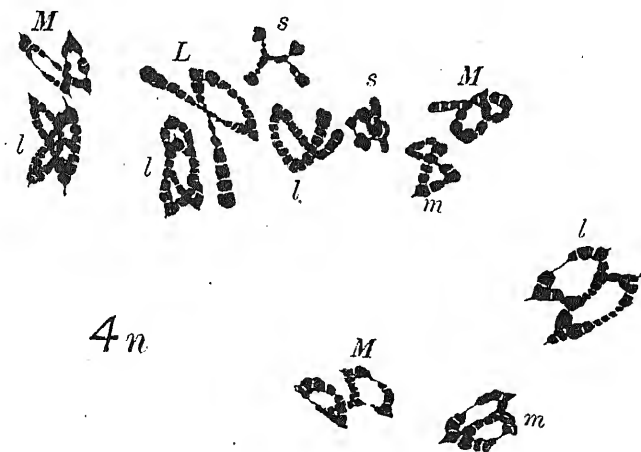
Fig. 7. Camera drawing of the quadrivalents of a true tetraploid at the metaphase of the first division in the pollen-mother-cell. The cytoplasm was squeezed from the cell and flattened out in close adhesion to the coverglass. The drawing was made with the preparation under a Zeiss 2 mm. apochromatic of 1·4 aperture, which was illuminated with a cone of light of over 1·2 N.A., from a Leitz aplanatic achromatic condenser corrected for water-immersion. A Wratten yellow-green light filter, No. 58, caused the carmine-stained chromosomes to appear black. These chromosomes were particularly distinct. The four chromosomes can be made out in all the quadrivalents, except perhaps the one marked *S*, which is somewhat folded. The six size-classes of the chromosomes are marked by the letters *L*, *l*, *M*, *m*, *S*, and *s*. Quadrivalent I, marked *L*, has the form of a ring and **V**. The four quadrivalents marked *l*, from left to right, are interlaced rings (two quadrivalents), bent chain of four, and double ring. The three *M*'s are double rings (two quadrivalents), and ring and **V** (or interlaced rings). The two *m*'s are probably both double rings. The *S* quadrivalent perhaps consists of interlaced rings; while *s* is a cross. There are several configurations of quadrivalents shown in Diagram 3 which are not represented in Fig. 5, but have been seen elsewhere. It is usually difficult to make out the nature of the quadrivalents in unsqueezed cells.

possible configurations have probably been seen, but some of them only once. There are eight of these (Diagram 3). It is probable that the double ring (No. 2) is the commonest, but the quadruple arc (No. 1) is often met with. No. 3, the triple arc and rod; No. 5, the ring with two rods; No. 4, the ring and V; and No. 6, the quadrilateral, or ring of four, are not often met with. No. 7, the four-rayed or X quadrivalent; and No. 8, the chain of four, are more often seen.

Quadrivalents (8)

Diagram 3.
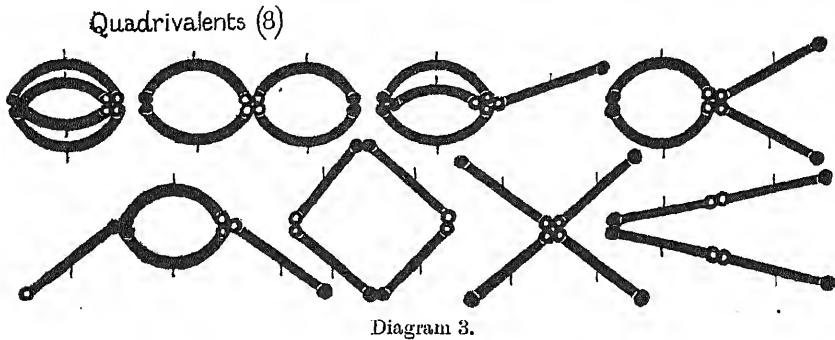
Taking one group of 12 quadrivalents, we find 12 free ends, 14 junctions of two ends, 4 junctions of three ends, and 11 junctions of four ends. There are thus only 11 junctions of four ends out of a possible 24. Hence, we may infer either that two combined chromosome ends exert less attraction than a single one, or that the junction of four is less stable than that of two ends, etc.

In the tetraploid Daturas a little over one quarter of the pollen-mother-cells show uneven distribution of chromosomes (Belling and Blakeslee, 1924). This is probably due to the fairly numerous cases of separation (non-conjunction). Twice the amount of non-conjunction as of true non-disjunction would of course be needed; and thus the former would occur somewhat under six times out of a hundred for *each* quadrivalent. It is, in fact, difficult to find a tetraploid group without some separate chromosomes. This large amount of separation again points to the inferior attractive powers of two or three combined ends as compared with an uncombined chromosome end.

TRIVALENTS OF SECONDARY $2n + 1$ MUTANTS.

(1) These forms are known to occur in Datura (Belling and Blakeslee, 1924 *b*) and probably also in Oenothera. Apparently only one or two, at most, come from each primary. In Datura, they have been found as

yet chiefly coming from the six primaries with the larger extra chromosomes, I to VI; there being few from the six primaries with the smaller extra chromosomes, VII to XII. Their configurations are intelligible only if we suppose that one of the three chromosomes of the trivalent has two homologous ends. Since a secondary arises repeatedly from its primary, and is the same every time, we must presume that the altered (or mutated) chromosome has undergone the same change in each case. Hence it perhaps arose from chromosomes divided at the constriction, for this is the only known definite point of fracture in the chromosome. Hence, probably by reversed crossing-over (reversed segmental interchange), either at the thin-thread stage, or later, two homologous chromosomes in a trivalent might interchange non-corresponding halves. Two mutated chromosomes, each with similar ends, and made of two similar halves, would result. This probably usually happens in a trivalent. If it happened in a bivalent, the two resulting gametes would probably not be viable in half the cases. There seems, too, no reason for such an abnormal position of two homologues in a bivalent; while, in the V trivalent of triploids and $2n + 1$ primaries, the two end chromosomes have been seen alongside in the late prophase nearly in the position necessary for such reversed segmental interchange. Hence secondaries could arise from the V trivalent. Since this configuration seems less frequent in the $2n + 1$ mutants with the extra chromosomes small, this may perhaps account for the infrequency of secondaries from these primaries.

In a trivalent containing two such altered chromosomes, if one of the changed chromosomes goes to the same pole as the unchanged one, then, and only then, there would be a gamete formed which would give a secondary on combining with a normal gamete. There should obviously be two kinds of secondaries for each primary, with different halves of the chromosome. When separate from the other two, which form a bivalent ring, the free chromosome seems nearly always to be the mutated one. This is doubtless a consequence of its being unable to form a bivalent ring with an unaltered chromosome, only one end of which attracts it. The altered chromosome also has a normal partner in the anaphase in probably two-thirds of the cases of (the ring of three) the V, and the chain of three. In all these cases secondaries are formed, not primaries. However, primaries might be formed in one-third of the cases of (the ring of three) the V, and the chain of three. In fact, secondaries are much more abundant in the progeny of secondaries than are primaries.

There are about seven possible configurations in the trivalents of

a secondary (Diagram 4). Six of these have been demonstrated with the microscope. The remaining one is the circlet and **V**, which may be presumed to exist from analogy with the **Y**, or three-rayed trivalent. The triple arc is not expected in a secondary, and does not occur. However, this is not of much significance, for in the primaries, where the
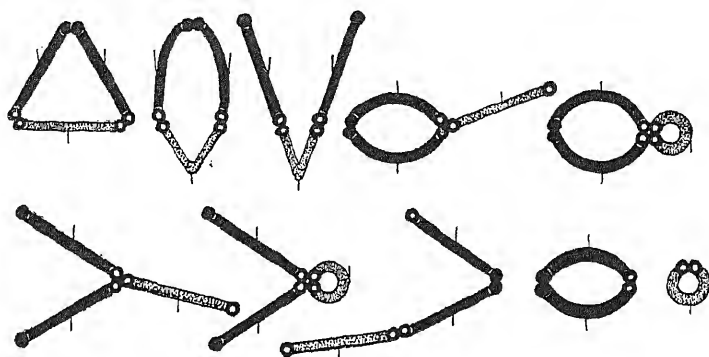
Trivalents of Secondaries (8)

Diagram 4.
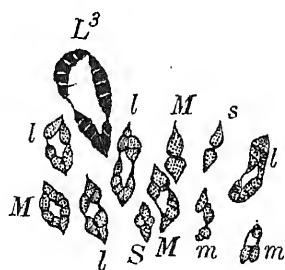
$$Sg.\ 2\,n + 1_{2a}$$

Fig. 8. Camera drawing of the first metaphase of the secondary $2n + 1$ mutant, Sg, with the altered chromosome in set I. The trivalent forms a closed **V**, or triangle.

triple arc is expected, it occurred in only one out of 108 trivalents. The trivalents of the secondaries differ mainly from those of the primaries (and, of course, from those of the triploids also) by having about one-half of their number in the form of a ring of three (or triangle) (see Figs. 8 and 9). This configuration does not occur in the primaries or in the triploids (compare Tables I and II). Also when one chromosome of
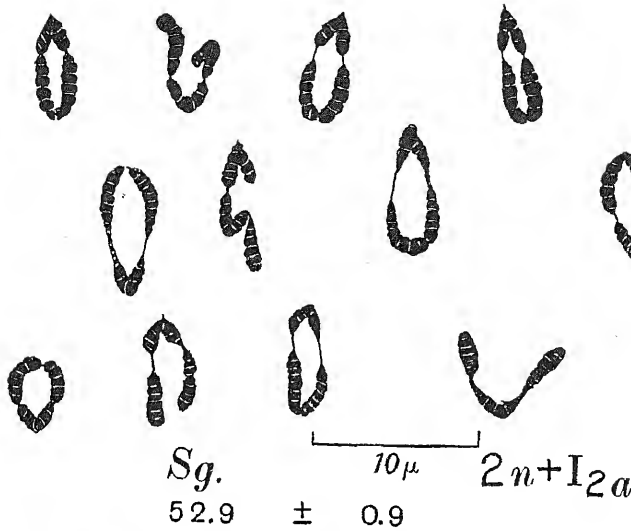
Fig. 9. These trivalents are from the first metaphase of the pollen-mother-cell in the secondary $2n+1$ mutant, Sg, derived from the primary, Rl, which is triploid for chromosome I. The extra chromosome, as the measurements show, is of the same size as chromosome I. But it has two homologous ends; so that, when alone, it bends into a small ring, the two homologous ends being in contact. When in a trivalent with two normal chromosomes of set I, it often forms triangles or closed V's. Thus in this sample of trivalents from Sg, there are seven closed V's, three open V's, one triangle, and one example of the ring and rod.

TABLE I.

Groupings of Chromosomes in Trivalents of Primaries.

Number of extra chromosome	Symbol of primary $2n+1$ mutant	Kinds of trivalents				
		Open V	Ring and rod	Y	Chain of three	Triple arc
I	Rl	6	3	—	2	1
II	Gs	3	2	—	—	—
III	Bk	9	2	—	1	—
IV	Ck	2	6	8	—	—
V	El	—	1	—	—	—
VI	Ec	9	2	1	—	—
VII	Mc	11	—	1	—	—
VIII	Rd	—	7	—	—	—
IX	Pn	3	—	—	—	—
(X)	(Sp)	3	1	8	—	—
XI	Gl	6	1	5	—	—
XII	Ix	3	1	—	—	—
	Totals	55	26	23	3	1

These trivalents include all that have been measured.

TABLE II.

Groupings of Chromosomes in Trivalents of Secondaries.

Number of extra chromosome	Symbol of secondary mutant	Kinds of trivalents			
		Closed V ring	Open V	Ring and rod	Y
(I)	*Sg*	8	3	1	—
(II)	*Sm*	3	2	—	—
(III)	*St*	3	1	—	—
(IV)	*Wd*	4	2	—	—
(VI)	*Mt*	7	3	1	1
	Sc	4	2	—	—
	Mp	1	5	2	—
	Totals	30	18	4	1

These trivalents include all that have been drawn and measured.

the trivalent is separate, this is nearly always the altered chromosome, as already stated. The altered chromosome has been seen in three of the secondaries in such cases, invariably rolled up into a circlet. Rarely the figure of eight, with a large ring of two homologous chromosomes and an attached circlet formed by the altered chromosome, has been demonstrated. The V, and the chain of three of the secondary differ presumably from the V and chain of three of the primary, in having homologous free ends. The fact that the Y is more common in the primaries than in the secondaries is perhaps due to the inclusion, among the ten primaries examined, of these with small extra chromosomes such as chromosomes X (primary, *Sp*), XI (primary, *Gl*) and XII (primary, *Ix*). In these small trivalents, V's may be scarce, or indistinguishable from Y's. Comparing the configurations in the primaries and secondaries from ten primaries (*Pn* and *El* being omitted), and seven secondaries, we have the following:

Ring of three	V	Ring and rod	Y	Chain of three	Triple arc	
—	48	33	17	9	1	108 from primaries
53	23	13	1	5	—	95 from secondaries

Reducing both to 100, we get this table:

Ring of three	V	Ring and rod	Y	Chain of three	Triple arc	
—	44	31	16	8	1	100 from primaries
56	24	14	1	5	—	100 from secondaries

The extra Y's in the primaries have already been accounted for. The extra V's and rings and rods are probably due to the absence of rings of three in the primaries.

The secondaries, of course, have many more junctions of two ends in their configurations than the primaries have. In the 108 trivalents

from ten primaries, there were 147 junctions of two ends; while in the 95 trivalents from seven secondaries there were 228 junctions of two ends. This excess was due, first to the many rings of three in the secondaries, each of which had three junctions of two, these rings of three being replaced in the primaries by the V, with only two junctions of two, or by the ring and rod, with only one junction of two chromosomes. Secondly, the many Y's, with no junctions of two, lowered the number of junctions of two in the primaries. But, as already noted, these Y's came partly from mutants with small chromosomes, not represented in the seven secondaries examined.

TABLE III.

Sizes of Chromosomes of Datura Stramonium, *Measured at the First Metaphase, in the Trivalents.*

No. of chromosome	Number measured	Length by average breadth	Symbol of $2n+1$ mutant	Size class of chromosome
I	36	54·9±0·9	*Rl*	*L*
II	15	44·8	*Gs*	*l*
III	36	41·0	*Bk*	*l*
IV	12	37·3	*Ck*	*l*
V	(3)	(35)	*El*	*l*
VI	36	32·1±0·3	*Ec*	*M*
VII	36	30·9±0·3	*Mc*	*M*
VIII	6	27·8	*Rd*	*M*
IX	9	22·9	*Pn*	*m*
X	15	22·6	*Sp*	*m*
XI	36	20·9±0·4	*Gl*	*S*
XII	12	18·7	*Ix*	*s*

TABLE IV.

Sizes of Chromosomes of Primaries and the Corresponding Secondaries.

Primaries			Secondaries		
Number	Symbol	Length by breadth	Number	Symbol	Length by breadth
I	*Rl*	55	(I)	*Sg*	53
II	*Gs*	45	(II)	*Sm*	43
III	*Bk*	41	(III)	*St*	48
IV	*Ck*	37	(IV)	*Wd*	40
VI	*Ec*	32	(VI)	*Mt*	32
	Total	210		Total	216

The greatest difference is that between *Bk* and *St*. The full number of 36 chromosomes has not yet been measured in *St*.

It will be evident from a glance at Table IV that the altered chromosome of a secondary is of the same size as the other two chromosomes of its trivalent, and as the three chromosomes of the trivalent of the corresponding primary (compare also Table III).

Hence, the only demonstrable difference in the configurations of the trivalents of primaries and secondaries is such as would be due to the presence of one chromosome with two similar ends in the trivalents of the secondaries.

Though the formation of such secondary mutants as these of Datura, and (probably) of Oenothera, does not appear to enter into the process of variety or species making; yet the phenomena are of interest to caryology as showing an unexpected mode of segmental interchange.

(2) In at least one case in Datura, and also in Oenothera (Miss Lutz, 1916), another kind of secondary has been found, in which there seems to be only one half a chromosome added to the normal diploid group, instead of two similar halves as in the true secondaries. The fracture (in the Datura mutant at least) took place at the median constriction. The configurations of the trivalent seen (and only a few were examined) were either the ring and rod, the half chromosome being the rod; or a **V**, the extra half chromosome being at one end of the **V**. Such a half chromosome should have one end not homologous with one end of the complete homologue; and so could not unite at both ends, but must always have one end free in the trivalent. It would then be at a free end in the **V** and the chain of three, and outside the ring of the ring and rod. Consequently it would nearly always have one of the normal homologues in the gamete with it, and should throw few or no primaries. Measurements, in one case in Datura, gave 29·8 (8 chromosomes) for the two large chromosomes of the trivalent, and 15·3 (4 chromosomes) for the half chromosome. This measurement of the half chromosome is less than the measurement of the smallest normal Datura chromosome, namely No. XII, which came to 18·7 (Table III). (In all these chromosome measurements, the product of length by average breadth is taken.)

These cases are perhaps chiefly of interest as bearing on the tertiary mutants, where the altered chromosome consists apparently of half (or other fraction) of one chromosome, and a complementary length of another, so that its size is (sometimes, at least) unaltered.

Trivalents and Quinquevalents of Tertiary Mutants.

So far, tertiary $2n + 1$ mutants have only been demonstrated to occur in Datura, though they probably came also in the second filial generation of species crosses of Stizolobium (Mucuna); where peculiarly leaved, nearly sterile, small plants were found in all crosses which included one species, in the proportion of about 1 in 200. The hypothesis

for the occurrence of semi-sterility in these crosses also accounted for these tertiary mutants (Belling, 1925 c).

In Datura, the tertiary mutant most studied, Wy, does not arise directly from a primary, as do the secondaries. It only arises from a certain primary (Pn, with extra chromosome IX, Fig. 10) after this

Pn

$2n + IX$
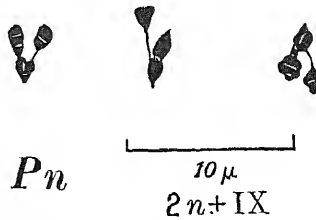
Fig. 10. This is a camera drawing of three trivalents of the $2n+1$ primary, Pn. The flowers of this primary are often abortive, and the chromosomes are hence difficult to obtain, without an abundance of material, which was not available. Hence these three trivalents are the only ones which could be drawn. The extra chromosome is IX. These chromosomes should be compared with the small chromosomes in Fig. 12. The size of the Pn chromosomes agrees with that of the small chromosome of Wy. The nine chromosomes of Pn averaged 23, while the 15 small chromosomes of Wy averaged 26 in size.

$2n + (IX_a I_c)_l$

Wy
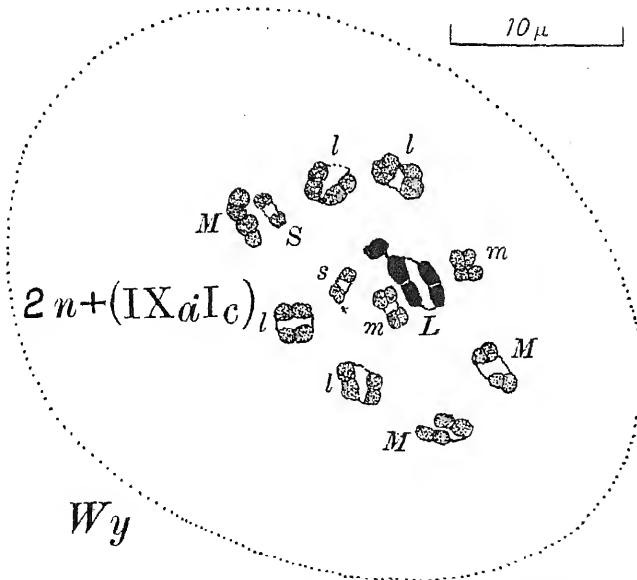
Fig. 11. Late prophase (diakinesis stage) of the tertiary $2n+1$ mutant, Wy. The trivalent consists here of two chromosomes of set I, joined to one chromosome of set IX. The chromosomes are *in situ* in the pollen-mother-cell.

primary has been crossed with an isomorphic strain of Datura, strain B. The lines which make up strain B apparently differ in no visible respect from the ordinary Daturas (though they happen to have white flowers). But they show abnormal ratios in flower colour when crossed with the primary mutants $2n + IX$, (Pn), and $2n + I$, (Rl). When crossed with

$$2\,n + \left(IX_a{\cdot}I_c\right) \quad Wy \qquad \overline{10\mu}$$
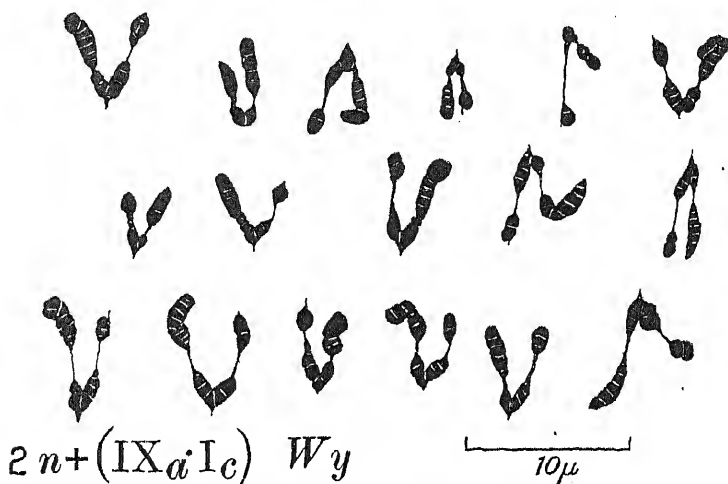
Fig. 12. These trivalents belong to the tertiary mutant, Wy. The abnormal chromosome ($IXa\ Ic$) came doubtless from the isomorphic B strain of *Datura Stramonium*. One of its ends is homologous with one end of chromosome IX. The other end is presumably homologous with one of the ends of chromosome I. There are over fourteen possible configurations, of which only two are shown here, though several others have been seen. (Three of these trivalents, Nos. 4 and 5 of the first line and No. 1 of the second line, were purposely taken from a small bud on a starved and unhealthy plant. They apparently show the minimum to which these chromosomes may be reduced by starvation, in cold weather.) The three starved trivalents, and also the second one on the first line, show two small and one large chromosome. The other 13 trivalents show two large chromosomes, equal to chromosome I in size, and one small chromosome, equal to chromosome IX. There have also been seen two trivalents consisting of three small chromosomes each; and a ring and rod, the ring being of two large, and the rod of a small chromosome. There have also been found, apparently, some chains of four and five chromosomes: but these cannot be certainly identified unless they are completely free from overlapping by the bivalents formed by the other chromosomes of the group.

The large chromosomes shown in this figure measured 54 as compared with 55 for chromosome I in Rl; and the small chromosome measured 26, as already stated.

Pn, they also cause the production of the tertiary mutant, Wy. This, as already stated, is the only tertiary which has been thoroughly studied; though several other $2n + 1$ mutants seem to belong to the same class, one of which, Hg, has been examined with the microscope more than the others.

The isomorphic B strain has apparently the same genes as the normal

white-flowered Daturas, so far as the tests show. It must then differ in the arrangement of those genes. A hypothesis which fits all the facts at present known is that there has been segmental interchange between the non-homologous chromosomes I and IX, in the ancestry of strain B. This is the same hypothesis, as already noted, which accounts for the semi-sterility of Stizolobium species hybrids, except that, in the case of Datura, the F_1 between the isomorphic line B and normals does not seem (always, at any rate) to be semi-sterile (Belling and Blakeslee, 1926).

In the mutant Wy, the majority of the trivalents seen are in the form of a **V**, with the small chromosome (the altered IX) at one end of the **V**, the other two chromosomes being the normal I bivalent (Figs. 11 and 12).

Configurations in Tertiaries
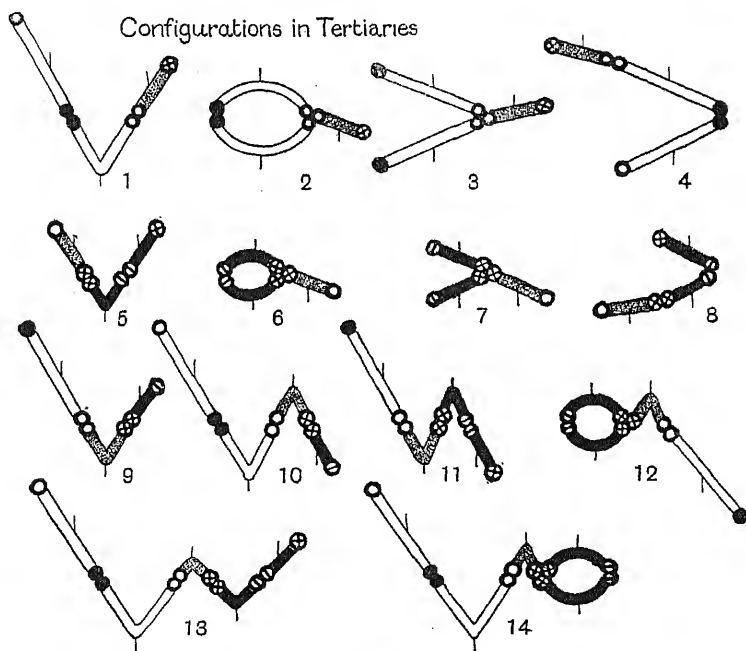
Diagram 5.

Less frequent was the ring and rod, the small altered chromosome IX forming the rod. Sometimes there were three small chromosomes in the trivalent, which was a **V**. Rarely two small chromosomes (IX and the altered IX) and one large one (I) have been seen. In the mutant Hg, quinquevalents (and quadrivalents) have been seen, as well as trivalents. They probably occur also in Wy, though they have not yet been demonstrated with sufficient certainty (compare Diagram 5).

The large chromosomes in the trivalents of *Wy* agree in measurement with chromosome I, and the small altered chromosome agrees with chromosome IX, as Table V shows. Hence the alteration has not

TABLE V.

Sizes of Chromosomes IX and I of Wy, *compared with those of* Pn *and* Rl, *respectively.*

Chromosomes	Length by breadth
IX or 9, from *Wy* (15)	26
IX, from *Pn* (9)	23
I, from *Wy* (27)	54
I, from *Rl* (36)	55

The numbers of chromosomes measured are in parentheses.

perceptibly affected the size of the chromosome. However, there is not merely a difference in the attraction of one end of this altered chromosome (in that it attracts one end of chromosome I, instead of a corresponding end of chromosome IX); but there is a difference in genes, since the tertiary *Wy* differs markedly in appearance from the primary *Pn*, $2n + IX$, from which it arises after crossing with the isomorphic *B* strain. The altered chromosome IX is then introduced from the *B* strain. *Wy* is a *Pn* with one chromosome of the trivalent altered.

Since the altered chromosome can combine at one end with chromosome I, and at the other end with chromosome IX, it may be presumed that it consists of a portion, perhaps one-half, of its length of chromosome IX, and the rest of a portion of chromosome I, equal to the missing part of chromosome IX. In other words, chromosomes I and IX seem to have undergone interchange of a terminal segment in the ancestry of the isomorphic *B* strain. (It is of course assumed that the altered chromosome I with a terminal piece of IX remains in the *B* strain, whose genes are thus presumably the same as in normals.)

It may readily be seen that this hypothesis would account for all the facts known, including the abnormal ratios for flower colour when the *B* strain is crossed with *Rl*, $(2n + I)$, and with *Pn*, $(2n + IX)$. For the genes for purple and white flower colour are in chromosome IX.

Since in the small V, of two normal IX chromosomes and one altered IX, the altered chromosome always goes with one of the normal chromosomes IX, there could be no primaries formed. For the gamete to form a primary, *Pn*, it must have two unaltered chromosomes IX. The same is the case for the larger V, of two chromosomes I and one altered chromosome IX. Some primaries, however, might be formed from
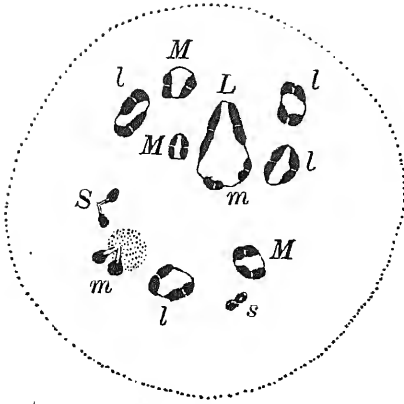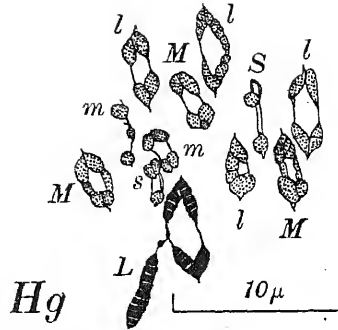
Fig. 13.   Fig. 14.

Fig. 13. Late prophase of first-generation plant of cross between the isomorphic strain *B* and a normal Datura. The quadrivalent is visible containing chromosomes Nos. I and 1 and Nos. IX and 9. There are ten bivalents also.

Fig. 14. First metaphase of the tertiary mutant *Hg*, showing the three large chromosomes in the form of a ring and rod.
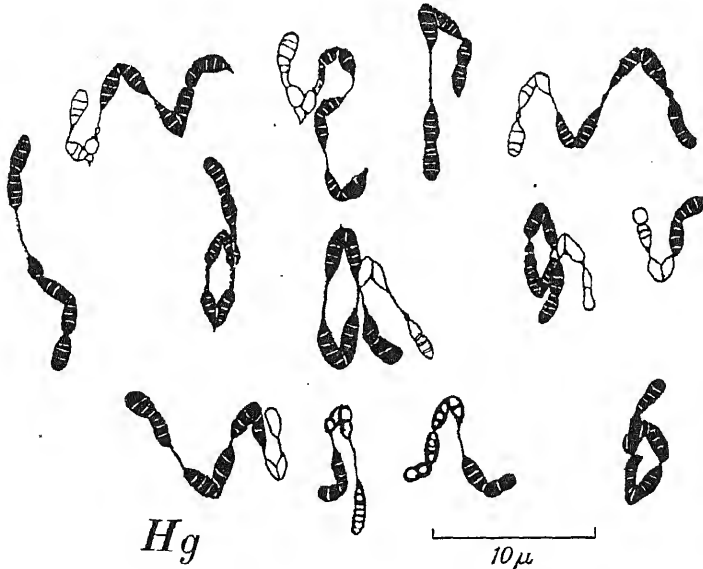
Fig. 15. This is a camera drawing of the chromosome configurations seen at the first metaphase in the $2n + 1$ tertiary mutant *Hg*. The large chromosomes are larger than chromosome I is in the mutants *Rl*, *Sg*, and *Wy*. The small chromosomes are nearly the same size as chromosome VI in the mutant *Ec*. There are six cases of three large and two small chromosomes, four cases of three large chromosomes, and three cases of two small and one large chromosome. Other configurations have been seen. The median constriction is visible in most of the chromosomes.

trivalents consisting of one chromosome I and two chromosomes IX, including the altered IX.

It may be deduced that chromosomes I and IX should form a quadrivalent at the reduction metaphase along with the altered chromosomes (1) and (9) in the first generation of crosses between normals and the isomorphic line *B*. These quadrivalents have been demonstrated with the microscope (Fig. 13). As might be expected, they are closed rings with the two short chromosomes together, and also the two long.

In the $2n + 1$ mutant *Hg*, the large chromosomes are apparently I, and the smaller chromosomes perhaps VI. One of the large chromosomes is probably altered (Figs. 14 and 15).

These tertiary mutants are perhaps of phylogenetic importance as showing probably segmental interchange of non-homologous chromosomes, and the origin of different isomorphic strains within a species, sometimes mutually more or less sterile (Belling, 1925 *c*).

Conjugation of Homologous Chromosomes.

This conjugation, effected at the thin-thread stage of the first maturation division, is not so readily studied in flowering plants as it has been in the Orthoptera, for instance. However, from an examination of the thick-thread and subsequent stages up to the first metaphase, probable deductions can be made as to the previous conjugation or non-conjugation of the homologues. For it may be concluded that homologues joined at both ends at the metaphase had previously conjugated. Of the homologues united at metaphase at only one end, it may be presumed from analogy that they were often so united at the thin-thread stages, and hence did not usually show true conjugation (parallel conjugation). This has been found in the tomato (Lesley, 1926). No doubt, at diakinesis or earlier, the connections between the homologues are made by difficultly visible threads, and perhaps some of these may be broken before metaphase. But, by analogy with the bivalents of diploids, such breakage should not be common, even in triploids and tetraploids.

(*a*) In the haploid Datura there is a thin-thread stage, with coiling chromosomal filaments. The thick threads which emerge from the skein are in loops, each of which forms a single chromosome. These *n* chromosomes show no attachments at the metaphase, and doubtless have not conjugated. The same is presumably the case with those separate chromosomes of diploids, etc., showing non-conjunction at the first metaphase.

(*b*) In the diploid Datura, each of the thick threads splits into two parallel loops which show chromomeres. The homologous ends of the threads are united by barely visible fibres. The pairs form loose rings at diakinesis, and close rings at the first metaphase. At diakinesis the homologues are still usually ill defined as compared with those of the first metaphase. In Datura there are nearly always 12 distinct rings at the first metaphase. In this case we may infer that conjugation occurred at the thin-thread stage. Sometimes, however, one pair of homologues is connected only at one end. It is possible that here conjugation did not occur; and at the thin-thread stage, the two homologues were, by a misadventure, only united at one end.

As already stated, in some few diploid plants (Tradescantia, and Oenothera), rings of four or more chromosomes are found at metaphase. It is possible that these chromosomes had not conjugated. In some diploids (such as Hyacinthus and Uvularia) with long chromosomes, crossing of the homologues at one, two, or three points perhaps results from conjugation and segmental interchange.

(*c*) In triploid plants, the triple arc, in which the three homologues are connected at both ends, doubtless comes from a triple conjugation. The ring and rod, on the other hand, probably, often or usually, comes from the parallel conjugation of two of the homologues, the third being connected only at one end (as M. M. Lesley, 1926, has seen in a triploid tomato). The **V** trivalent, possibly, had undergone no true conjugation.

(*d*) In tetraploids, the quadruple arc doubtless results from the conjugation of four homologues together. The triple arc and rod presumably arose from a triple conjugation. But the common double ring probably came from two pairs of homologues conjugating two by two. Two other forms show one pair of conjugants. The square, the cross, and the chain of four, have retained no indication of conjugation.

(*e*) In the secondaries, the figure of eight doubtless arose from the conjugation of the two homologues, and also perhaps the conjugation of the two halves of the mutated chromosome. The latter also probably took place in the free circlet. In the triangle (or closed **V**) no indications remain.

(*f*) In tertiary mutants, the ring and rod, where the mutated chromosome is the rod, doubtless had undergone conjugation of the two homologues. Most of the other forms show no signs of conjugation having occurred.

(*g*) The chromosomes of the ring quadrivalents formed in the F_1 of

the cross of two isomorphic lines probably did not conjugate, or perhaps only conjugated partly.

<div align="center">SEPARATION OF ATTACHED CHROMOSOMES.</div>

There are various ways in which it may be determined how the homologues separate. (We will disregard here plants which show chromosomal junctions at other points than the ends, and so at the metaphase may show separation of the chromatids of parts of the homologues.) The following criteria are usually available to show to which pole any chromosome of a configuration is about to pass, at the first metaphase or early anaphase. (1) The apex of any sharp bend in the chromosome points to the pole to which it is being pulled. (2) A short stained thread points directly to the pole, being at the base of the spindle fibre. (3) The point of constriction of the chromosome is nearest to the pole to which the chromosome is proceeding, for the spindle fibre arises here. (4) A taut fibre between adjacent chromosomes of a configuration, usually points along the direction in which these chromosomes are proceeding. (5) The deflection of an attached straight terminal chromosome from the horizontal, points to the pole it is nearing.

(*a*) In the haploids the chromosomes are already separate, and are pulled at random to the poles without deformation. (*b*) In the diploids with short chromosomes the random orientation of the bivalents with regard to the two poles does not explain why both spindle fibres from any bivalent do not pass to the same pole in half the cases, but always apparently go to opposite poles. Homologues accidentally connected at only one end keep straight as they are separated and pulled to opposite poles. (*c*) In triploids, two chromosomes of the triple arc go to one pole, and one to the other. The two halves of the ring in the ring-and-rod trivalent pass to opposite poles, and the rod goes to one pole at random. In the V, the two end chromosomes pass to the same pole, and the bent chromosome to the other pole. Also in the Y and the chain of three, two chromosomes pass to one pole, and one to the other. (*d*) In tetraploids, two of the chromosomes of the quadruple arc seem to pass to one pole and two chromosomes to the other pole. Similarly with the double ring, the square, the cross, and the ring and V. The triple arc and rod, the ring and two rods, and the chain of four have more than one possible distribution of the homologues. (*e*) In the secondaries, the closed V is usually orientated as if the bent chromosome passed to one pole and the two others to the other pole. In the figure of eight, the circlet accompanies one of the non-mutated chromosomes, so that secondaries

and not primaries would be formed. (f) In tertiary mutants, the mutated chromosome, being usually at one end of the open V, or the rod of the ring and rod, passes to a pole with one of the non-mutated chromosomes of the same kind; either from the same trivalent, or from a separate bivalent. Much the same happens in the quinquevalents, where alternate chromosomes pass to opposite poles (as in the compound rings of some Oenotheras, Cleland, 1924).

SUMMARY.

(1) In the haploid plant the n chromosomes show no mutual attractions at the first metaphase, their distribution being a random one. This proves that the attractions of the $2n$ ends of these n chromosomes for their homologues are all different. (2) In most diploid plants the $2n$ chromosomes form n rings attached at both ends. Trivalents or other multiple configurations are found. When the constriction is subterminal it may be seen that the homologous ends of the homologues come together. This shows that the attractions of the two ends of a chromosome are different. Hence there are $2n$ different kinds of attraction. (3) In some diploid plants (such as Hyacinthus and Uvularia) the homologues are sometimes attached at the constrictions. This brings in n (or perhaps $2n$) more, presumably different, attractions. (4) In diploids with long chromosomes the homologues are attached at other points. These are possibly consequences of segmental interchange. (5) In triploids, the union of three homologous ends is not so common as that of two. Thus the union of two ends in some way hinders union with a third homologous end. (6) The absence of a triangle among the configurations of trivalents in triploids proves again that the attractions of the two ends of any normal chromosome are different. (7) All the five configurations possible on the hypothesis (of $2n$ different attractions) have been found in triploids. (8) In tetraploids, unions of two or three homologous ends are more common than those of four ends, showing again that the attraction is lessened after union. (9) All the eight configurations expected on the hypothesis have been found in tetraploids and only those eight. (10) In secondary $2n + 1$ plants, one of the chromosomes of the trivalent has the same attraction at both ends; for when separate its ends meet to form a circlet. Genetic considerations show that it probably consists of two homologous halves. (11) The triangle occurs frequently in a secondary, but not in a triploid or in a primary. This again proves that one chromosome of the secondary has similar ends. (13) The mutated chromosome possibly arose from reversed segmental interchange of halves

(at the constriction) in the V trivial of a primary where two homologues are parallel in a reversed position, and the genes are identical only at the middle. (14) In one tertiary $2n + 1$ mutant, one of the chromosomes of any trivalent, etc., attracted at one end the corresponding end of its homologue, while at the other end it attracted one end of a certain non-homologous chromosome. (15) In consequence, one end of this mutated chromosome, 9, was combined with two chromosomes IX to form a small V trivalent; or the other end was combined with two large chromosomes I to form a large V trivalent (or possibly quadrivalents or quinquevalents might occur). (16) In this tertiary mutant, measurements of chromosomes and genetic results lead to the conclusion that one-half of chromosome IX had interchanged in its ancestry in the mutated chromosome with an end-piece of the long chromosome I. (17) This tertiary mutant arose after crosses of two isomorphic strains of *Datura Stramonium* (the normal and strain *B*). Strain *B* gave, in conformity with the hypothesis, abnormal ratios when crossed with the mutants $2n + I$ and $2n + IX$, for genes in IX. (18) Crosses of the normal and strain *B* showed, as was expected, quadrivalents consisting of chromosomes I and 1, and IX and 9 in a closed ring (1 and 9 being the two mutated chromosomes found in strain *B*). (19) Thus the occurrence of isomorphic strains points to the occasional occurrence of crossing-over between non-homologous chromosomes, as to which caryological data are lacking. (20) A $2n + \frac{1}{2}$ mutant, in which a half chromosome is extra, had trivalents showing junctions only at one end of the half chromosome and the normal homologues. This shows again that a chromosome has a different attraction (if any) at the constriction. (21) From the configurations at the first metaphase more or less probable inferences can be drawn as to the presence or absence of conjugation at the thin-thread stage. (22) Several criteria permit the separation of the homologues at anaphase to be inferred from the configurations.

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GENETIC STUDIES IN POULTRY. V. ON A CASE OF PIED PLUMAGE.

By R. C. PUNNETT, F.R.S. and M. S. PEASE, M.A.

(With Four Text-figures and One Plate.)

In Report IV to the Evolution Committee of the Royal Society (1908) a brief account was given of the genetical behaviour of fowls showing a mixture of white and colour in the plumage, and it was shown that this "mottled" condition, as it was termed, behaved as a simple dominant to self-colour. In the course of our experiments with poultry we have recently come across another case in which a mixed coloured and white plumage also presents to self-colour a simple Mendelian relation. In the present case however the mixed type of plumage, which we shall term "pied," behaves as recessive to self-colour. The data are not extensive, since, the experiments from which they were taken were designed for the solution of other problems. Nevertheless the conclusion to which they point is sufficiently obvious, and as it is now clear that we must reckon with two types of mixed plumage, not dissimilar in appearance though genetically quite distinct, we consider it worth while placing the facts on record.

The pied birds in question were met with in the F_2 generation from a cross between a Barred Rock ♀ and a Black Sumatra ♂, of which the relevant details may be gathered from the pedigree on page 208. As was to be expected from such a cross the F_1 ♀♀ were all black and the F_1 ♂♂ were all barred. In many cases these birds put up a few white feathers, but as this frequently occurs in black breeds and as it had no bearing on the enquiry for which the experiments were designed, no special note of it was made at the time.

In 1925 two F_1 cockerels (♂ 266/24 and ♂ 369/24) were mated with three and with four sister birds respectively, while a third F_1 cockerel (♂ 192/24) was run with three pure Sumatra pullets, all daughters of the original Sumatra cock. From each of these pens some F_2 birds, chiefly pullets, were reared to maturity, and in each case a few of them turned out to be pied (cf. Plate X, figs. 1–4). From the two F_2 cockerels run with sister birds only one pullet in each case definitely produced pied birds, viz. ♀ 323 and ♀ 372. On the assumption that the

PEDIGREE.

♂ P.R.

♀ 6/24
P.R.

[−18/24]
×

[−18/24]
×

♀ 8/24
Sumatra

♂ 9/24
Sumatra

♀ 323/24 ⎱ [−51/25]
♀ 397/24 ⎰ × ♂ 266/24
♀ 451/24

[3 Pied
25 not Pied]

♀ 359/24 ⎱ [−45/25]
♀ 372/24 ⎰ × ♂ 369/24
♀ 11/24

♀ 356/24 [2 Pied
17 not Pied]

♂ 192/24 [−49/25] ♀ 548/24
× ♀ 549/24
♀ 550/24

[5 Pied
15 not Pied]

♀ 187/24 [−25/25] ♂ 5/24
×

♂ 269/25 [−17/26] ♀ 113/25 [−18/26] ♂ 9/24 (Sumatra)
× ×
[11 all Pied] [1 Pied]
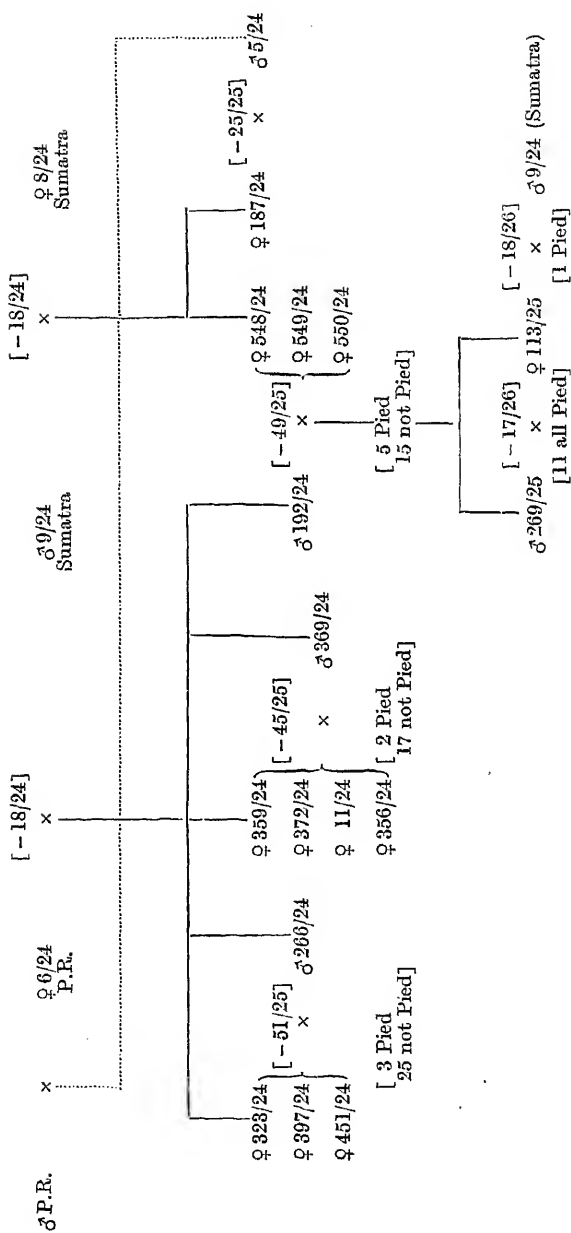
pied condition is a simple recessive we may suppose that in these two pens of F_1 birds the two cockerels and the two pullets mentioned were heterozygous, while the remaining five pullets were probably homozygous blacks. The third F_1 cockerel ($\male$ 192), which was run with Sumatra pullets, produced 15 blacks and 5 pied chicks. He himself must therefore have been heterozygous. In this case the offspring from the three pullets were not separately recorded so that we cannot positively assert that more than one of them was heterozygous.

In the following year (1925) a pen was made up with a pair of these pied birds, viz. $\male$ 269 and $\female$ 213, and the 11 offspring reared from them all proved to be pied.

Since the original parents for the Rock × Sumatra mating were still in existence we attempted to decide from which one the pied character had come into the cross. Accordingly the Rock hen ($\female$ 6) and the Sumatra cock ($\male$ 9) were each·mated with a pied bird. These matings were made rather late in 1926 and the Rock hen unfortunately ceased to lay, but from the Sumatra cock a few chicks were obtained. The only one we succeeded in rearing turned out to be pied, showing that our original Sumatra $\male$ was heterozygous, and that the pied character had come into the cross through him. Though the original Rock hen was not directly tested, the fact that some two dozen birds were reared from her by the Sumatra cock and that there was not a pied individual among them makes it practically certain that she did not carry the pied character.

The data then are consistent with the view that the pied condition behaves as a simple recessive to self-colour, and that it was introduced into the experiments through our original black Sumatra cock.

Further evidence of the recessive nature of this pied character is afforded by the following considerations. The mottled appearance of the plumage is brought about partly by the whitening of the tips of the feathers, and partly through the pure white feathers interspersed among the rest (cf. Text-fig. 1). Birds may be described as light or dark pied according as the white markings on the feathers tend to be more or less pronounced, and according as the pure white feathers themselves tend to be more or less numerous. Also a bird looks lighter when the pied character is associated with the Rock type of barring than when the same grade of piedness is on a self-black basis. When the first pied birds appeared in our matings we were at once struck by their resemblance to a breed known as the "Exchequer Leghorn"; and in respect of this plumage character we were prepared to find this breed

behaving as a recessive to the black. Through the kindness of Capt. F. S. Morgan we have been able to obtain experimental data in support of our supposition. Capt. Morgan mated Black Leghorn ♂ with Exchequer Leghorn ♀♀ and obtained only blacks in F_1[1]. These black pullets when mated back to an Exchequer ♂ gave Exchequer-marked (pied) birds and blacks in approximately equal numbers. We do not therefore feel any doubt that the Exchequer Leghorn is a recessive pied bird, genetically similar in respect of this character to the pied birds which turned up in the course of our own experiments.
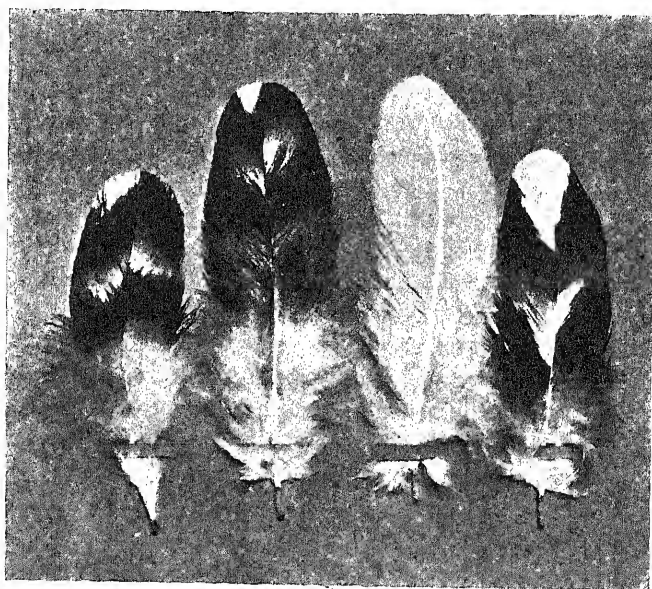
Text-fig. 1. Showing some feathers from a barred pied bird.

All the available evidence then is in favour of the view that this type of pied plumage behaves as a simple recessive to full colour, and we may now consider briefly our data relating to the downs. Here the facts are more complicated, and before setting them out we may make a few general remarks in connection with the downs of black fowls. As is well known to breeders, the chicks of self-black breeds are black in the down, but always show some creamy white on the ventral surface. The extent of this ventral white exhibits considerable variation.

[1] The pullets were said to be full blacks, while the cockerels tended to show some white in the tail.
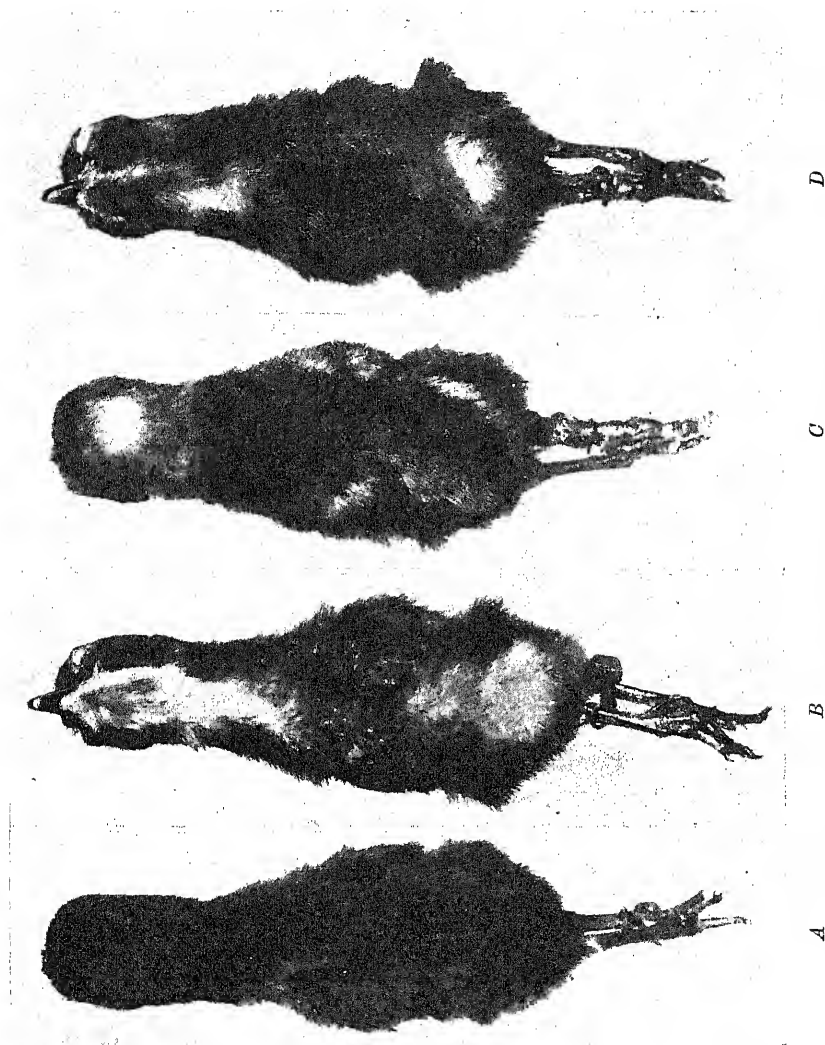
Text-fig. 2. Showing dorsal (*A*) and ventral (*B*) surfaces of down in black chick, and similar views (*C* and *D*) in a "barred" chick with a well-marked light head patch.

Generally speaking it is not visible when the chick is viewed from the dorsal surface (Text-fig. 2). But in some breeds there is a marked tendency for this ventral white to increase in amount, to creep, as it were, up the sides, and eventually to invade the dorsal surface, especially

A   B
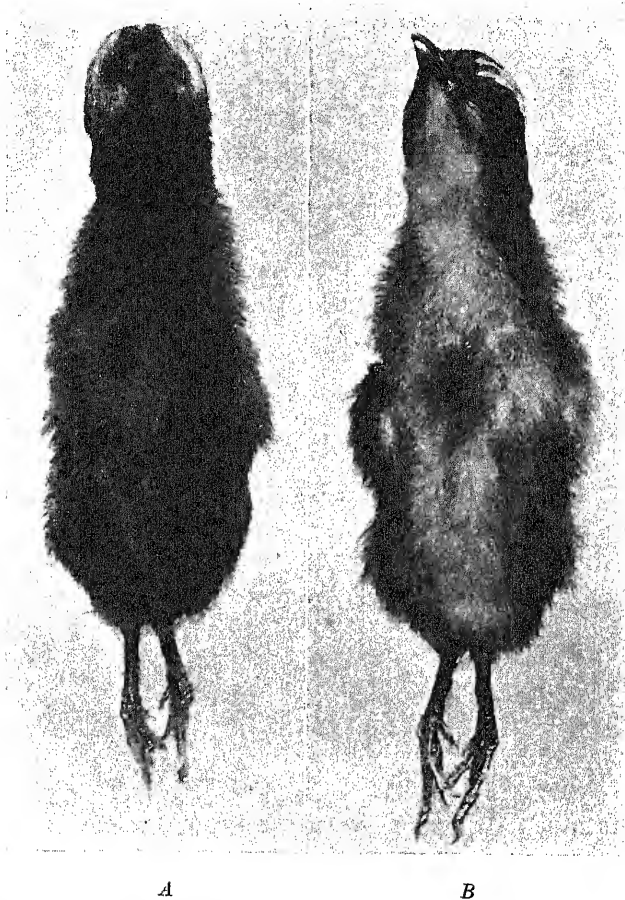
Text-fig. 3. Down of black chick with rather more white than usual. The white tends to invade the dorsal surface (*A*) of the head.

in the region of the head and neck (Text-fig. 3). Such a tendency to an excessive amount of white in the down we have noticed especially in Black Rosecomb Bantams, Black Sumatras, and Black Leghorns; and it is possibly not without significance that in these varieties the breeder has sought a high degree of beetling in the plumage, and at the same

time is inclined to regard leniently the presence of an occasional white feather. It will of course be understood that we do not wish to imply that a large amount of white in the down, or the reverse, is a breed characteristic. On the contrary it is more a matter of strain, or even of individuals in a strain. Black Leghorns may show as little white as, for example, Black Langshans. But in our experience much white in the down is relatively more often to be met with in the former breed than in the latter, and indeed is more or less characteristic of some strains of Black Leghorns. We are inclined to suspect that it is associated with enhanced beetling, and that in selecting for this last character the breeder has at the same time unconsciously selected for an increase of white in the down.

Our own interest in the matter arose from the fact that the excessive amount of white in the down which appeared in the course of our Sumatra-Rock experiments tended, by invading the head and neck region, to render it difficult, or even impossible, to determine the presence or absence of the light occipital head spot through which the barred may be distinguished from the self-black class at hatching. Since then we have learned that several breeders, making use of the cross Barred ♀ × Black ♂ to give a sex-linked result, have met with the same difficulty. Although we have not been able to carry out a series of experiments

TABLE OF MATINGS.

			Norm.	"White"	Remarks
			Downs	
1924	Pen 18. 6	P. Rock ♀ × Sum. ♂	25	18	"Whites" here towards normals, *i.e.* not of high grade.
	„ 18. 8	Sum. ♀ × Sum. ♂	—	18	"Whites" vary from a little white on head to much white.
1925	„ 25. 187	Sum. ♀ × P. Rock ♂	33	11	"Whites" here of low grade, *i.e.* not enough white on head to disguise the light head patch.
	„ 49	Sum. ♀♀ × F₁ ♂	17	16	Includes 5 pied birds with much to excessive white.
	„ 45	F₁ ♀♀ × F₁ ♂	54	14	After deducting 4 "whites" as pied birds.
	„ 51	F₁ ♀♀ × F₁ ♂	58	22	After deducting 6 "whites" as pied birds.
1926	„ 17	Pied ♀ × Pied ♂	—	23	"Whites" here all of high grade, *i.e.* with much to excessive white.
	„ 18	Pied ♀ × Sum. ♂	—	6	Five with much white of which one pied; one with "some white" on head.

sufficiently extensive to enable us to offer a complete analysis of the various grades of down we think that what we have been able to learn will probably prove helpful in connection with this difficulty.

In the table on page 213 we have set out the relevant facts as gathered from our note-books of 1924–26, but before discussing them in
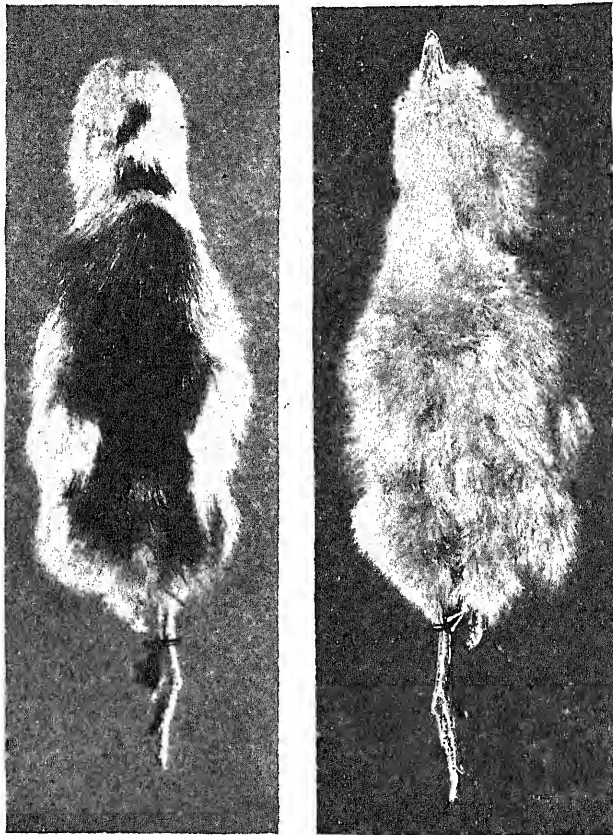
<center>A   B</center>

Text-fig. 4. Down of "pied" chick showing dorsal (*A*) and ventral surface (*B*).

detail we may briefly outline the interpretation that we would suggest. In the first place the pied plumage has always been associated with much white in the down, and the lightest downs that we have met with in the course of our work have occurred among chicks that eventually developed the pied plumage (Text-fig. 4). Nevertheless chicks

of a light type may develop into normal blacks or barreds. Most of our pure Sumatra chicks, for example, showed an unusual amount of white in the down, though eventually they developed into birds with normal black plumage. The amount of white was variable, some chicks showing a good deal more than others. With sufficient experimental work this variable series could doubtless be expressed in terms of a few definite genetic factors such as we have already determined in the case of the Dutch rabbit[1]. Since, however, our chief interest lay in another direction we have not attempted the long and laborious analysis involved, and consequently the explanation that we offer must be in more general terms than one based upon a complete factorial analysis of the various grades of pigmentation involved.

We suggest that the difference between a normal black down and one with an unusual amount of white[2] is primarily due to a factor P. PP chicks have normal black downs, while pp chicks exhibit an unusual amount of white. The heterozygous (Pp) chick may either have a normal black down, or one with rather more white than usual, according to its constitution in respect of some modifying factor or factors whose existence is here postulated on analogy with other and more fully investigated cases of a similar nature. Thus pp chicks, as mentioned above in the case of the pure Sumatras, vary considerably in the amount of white in the down, and it is these variations that we regard as due to the modifying factors postulated. A very light pp chick crossed with a normal might be expected to give Pp chicks with more white than in the normal; whereas a pp chick from the darker end of the series when mated to a normal would probably give Pp chicks in which the dominance of P was almost or quite complete.

The second factor entering into the case is that upon which depends the difference between a self-black and a pied bird. When this factor (E) is present the bird may have either a normal down or one with more white according as it does or does not contain P. But pied birds (ee) always show much white in the down however they may be constituted in respect of P. Further, it would appear that we have no grounds for supposing that there is any difference in appearance between EE and Ee chicks provided that they are of similar genetical constitution in other respects.

We may now revert to our Table (p. 213) and enquire how far this

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[1] *Journ. Gen.* xv. 1925.

[2] A part of course from the "white" downs characteristic of chicks that develop into pied birds.

hypothesis is compatible with the data there set out. It clearly accords with the fact that all of the pure Sumatra chicks (Pen 18, 1924) showed an unusual though variable amount of white as compared with normal blacks, and we must suppose both parents to have been pp in constitution, though at the same time probably differing in the postulated modifying factors.

The result of crossing the Rock hen with the Sumatra cock (Pen 18, 1924) was to give 25 chicks with normal black (or barred) downs and 18 with distinctly more white than usual, though not on the average as much as in the pure Sumatra. Since all of these F_1 chicks must be regarded as Pp we suppose those showing more white to correspond with the lightest downs in the pure Sumatra, and those with normal or almost normal black down to correspond with the darker forms of down in that breed. And here we may remark that since the Sumatra cock used proved to be heterozygous for the pied character (Ee) it seemed possible that the F_1 chicks with more white might be Ee, and the normal blacks EE in constitution. This possibility is however negatived by the fact that both kinds of down occurred in F_1 chicks subsequently shown to carry the pied character, as well as in those which proved to be homozygous for E[1].

The results from the F_1 birds bred together (Pens 45 and 51, 1925) are complicated by the fact already noted that both of the cocks, and one pullet in each pen, carried the pied character. Since pied birds from these pens may be either PP, Pp, or pp in constitution, and since they always have the whiter type of down, it is clear that they should be left out of account in attempting to estimate the ratio of downs with and without P. The eggs of only two birds are concerned, viz. ♀ 323 and ♀ 372, but in neither case is it possible to decide exactly how many of the white type of downs would have become pied birds. In part this is because some chicks died shortly after hatching, and in part because in each pen some eggs were not definitely allocated to a particular bird. Taking into consideration these facts we have calculated the number of chicks which, including those that were actually pied, should be reckoned as pied birds. Without going into the details of our calculations we may say that of these there were four in Pen 45 and six in Pen 51, and we have accordingly deducted them from the totals. After this correction

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[1] Of the five F_1 birds carrying pied one (♂ 192) had normal black down, while the rest (♂♂ 266, 369, ♀♀ 323, 372) had rather more white than usual: of the five F_1 birds homozygous in E two (♀♀ 11, 356) had normal black down, and three (♀♀ 359, 397, 451) had rather more white than usual.

the figures for the two pens together are 112 normal black downs and 36 with an excess of white. Since the parents must all be supposed to have been Pp in constitution such an approximation to a 3 : 1 ratio is what would have been looked for on our hypothesis.

The same difficulty in connection with the pied chicks occurs also in the back cross of the F_1 ♂ 192 on to the Sumatra pullets (Pen 49, 1925). The downs as recorded are 17 normal black and 16 of a whiter type where equal numbers of the two are to be looked for. Since only Ee and ee chicks occur the simplest way of correcting is to deduct the pied birds, leaving 17 normals and 11 with much white—a slight excess of normals over expectation, but one on which much stress cannot be laid in view of the small numbers involved.

The last mating which calls for consideration is that between a Sumatra pullet and a Rock cockerel (Pen 25, 1925). This is recorded as having given 33 normal black and 11 rather whiter downs. In these last, however, the excess of white was small, and not enough to interfere with the recognition of the light head patch characteristic of barred chicks.

Regarding the evidence as a whole, and allowing for the probable existence of a factor or factors tending to produce minor modifications in the extent of the white, it would seem to accord reasonably well with the hypothesis suggested; though at the same time we recognise that the relations between the various grades cannot be precisely expressed without a more lengthy analysis than we were prepared to undertake. Incomplete, however, as our analysis is we have put it forward because we feel that it may be of some value to those who make use of the phenomenon of sex-linkage in breeding poultry, and encounter the difficulty of an excessive development of white in the down obscuring the light head patch characteristic of the male chicks from the cross between barred ♀ and black ♂. It is now clear that this difficulty may be due to two quite distinct causes. In the first place it may arise through the crossing of birds which both carry the recessive pied character. Such a result is more likely to be met with in the cross between Cuckoo and Black Leghorns, in which race the pied condition is recognised as a distinct variety under the name of the "Exchequer Leghorn." Owing to its simple recessive nature the pied character can be readily eliminated in the usual way. When, however, chicks with much white appear in such a cross as Rock ♀ × Black Leghorn ♂ it is more likely to be due to the Leghorn belonging to a strain with a low grade of pigmentation in the down. We suspect this to be associated with a high

degree of beetling in the adult plumage such as is encouraged in show birds, but whether this be so or not the breeder who wishes to make use of such a sex-linked cross would be well advised in selecting his black cocks to avoid a strain in which an excessive amount of white occurs in the down of the chicks.

SUMMARY.

The "pied" character, a mixture of coloured, particoloured and pure white feathers (such as occurs in the Exchequer Leghorn), behaves as a simple recessive to self-coloured plumage. Pied chicks show a great deal more white in the down than normal blacks.

An excess of white in the down may however be quite independent of the pied character, occurring in chicks which develop into normal blacks. Such "white" downs are recessive to normal black downs though the grade of pigmentation is probably complicated by a modifying factor, or factors.

The bearing of these facts is discussed in connection with certain difficulties sometimes met with in making use of the sex-linked cross barred ♀ × black ♂.

EXPLANATION OF PLATE X.

Photographs of four pied pullets of which 1 and 2 are unbarred, and 3 and 4 are barred. Figs. 1 and 3 represent the most heavily pigmented, and figs. 2 and 4 the least heavily pigmented forms that occurred in our experiments.
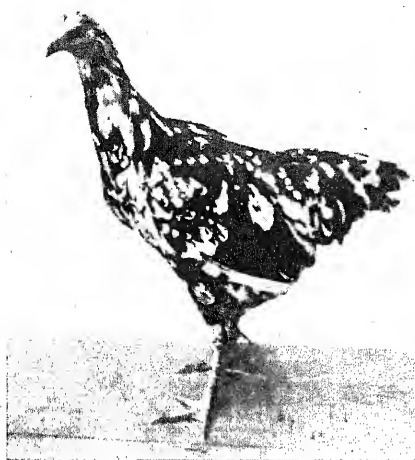
Fig. 1.
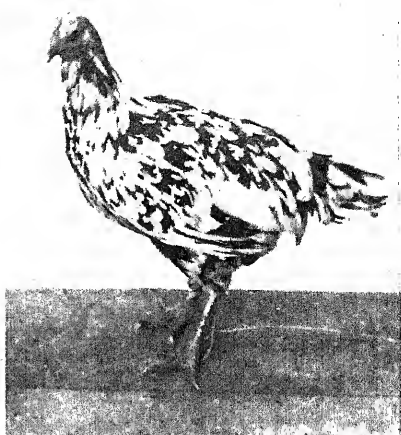
Fig. 2.
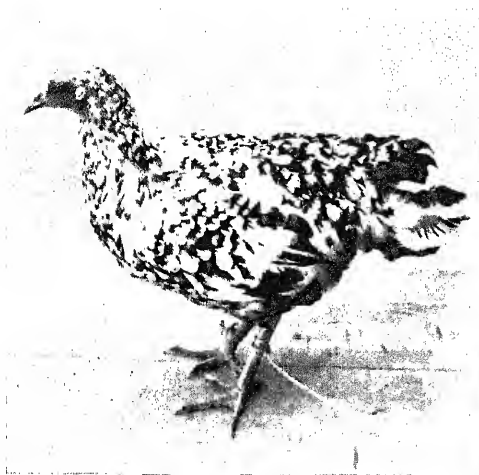
Fig. 3.

Fig. 4.

DOMINANT BLACK IN CATS AND ITS BEARING ON THE QUESTION OF THE TORTOISESHELL MALES—A CRITICISM.

By RUTH C. BAMBER (Mrs BISBEE), M.Sc., F.L.S.
AND E. CATHERINE HERDMAN, M.Sc.

In the *Journal of Genetics* for October 1926, vol. XVII, no. 2, pp. 207–209, K. Tjebbes and Chr. Wriedt call attention to the occurrence of a dominant black in cats and suggest that it is the key to the much-discussed problem of the tortoiseshell male.

Ordinary black in cats is recessive to the ticking which gives tabby. Tjebbes (1924) crossed a tabby male with a Siamese female and obtained all blacks in the F_1 generation. These blacks crossed *inter se* gave, amongst others, two tabby kittens. The black of the F_1 generation was, therefore, obviously *dominant* to tabby. Tjebbes and Wriedt now give the results of three additional matings of the same type, and these confirm the occurrence of this dominant black.

They also record the mating of a Siamese female with a "striped yellow male" and say that three tortoiseshell kittens were produced, two males and one female. On the strength of this result they suggest that dominant black is probably the explanation of all tortoiseshell males.

The tortoiseshell male is of outstanding interest in regard to the problem of the inheritance of coat-colour in cats and also in connection with the larger question of the chromosome theory both of inheritance and of sex-determination. It is, therefore, especially desirable that any theory of his origin should be examined with the greatest care. When so examined, the suggestion of Tjebbes and Wriedt does not seem to be in harmony with the facts of the case.

From Tjebbes' own experiments it is clear that his dominant black is not affected by the factor for ticking, which gives tabby. If tortoiseshell males were the result of the combination of yellow and this dominant black, it should be impossible ever to get one with his black changed to tabby, yet such a tabby-tortoiseshell male is at present in our possession. His coat is a mixture of yellow and tabby arranged in irregular patches, with white chest and feet. Such tabby-tortoiseshells are common amongst females, but males are extremely rare. Fanciers refer to them

casually, but only one other, to our knowledge, has been definitely recorded. It is practically certain that the only difference between a black and a tabby lies in the presence of a factor for ticking (Doncaster, 1913, p. 21 footnote; Whiting, 1918 and 1919; Bamber, 1927, pp. 5–13), so that our male is a tortoiseshell with the ticking factor added.

It is, of course, possible that two doses of ticking might give tabby with one of dominant black although one dose, as in Tjebbes' results, has no visible effect, but it is unlikely that the "tabby" so produced would be indistinguishable from normal tabby. The fact that the large patches of tabby on our male are perfectly normal suggests that dominant black is not the key to his exceptional colouration. He is fertile but has been in our possession for a short time only and so far his offspring[1] throw no light on the present subject.

Another point in Tjebbes' and Wriedt's paper calls for comment. They record two tortoiseshell males and one tortoiseshell female from the cross yellow ♂ × Siamese ♀. But a yellow male does not normally transmit yellow to his sons—yellow being apparently sex-linked (Little, 1912 and 1919; Doncaster, 1912 and 1913, p. 19; Bamber, 1927, p. 43; Bamber and Herdman, 1927, pp. 88–94). Here he appears to have done so in two cases in one litter. In view of the extreme difficulty of sexing some kittens at birth, it will not be considered unduly critical if we express a hope that these recorded tortoiseshell males will either be allowed to grow up, or be dissected. Some kittens are quite unmistakably male, others as unmistakably female, but some are so very indefinite that, even with kittens of both sexes in the same litter for comparison, it is not always possible to say to which sex they belong. There is therefore a possibility that if Tjebbes' and Wriedt's kittens are still young, they *may* have been sexed incorrectly. We dare to suggest this only because the occurrence of *two* males in one litter both inheriting yellow from their father is such a very extraordinary result.

Taking for granted, however, that the sexes are correctly recorded, the occurrence of the two tortoiseshell males is no justification whatever for the assumption that dominant black is the key to the problem. These males got their yellow from their father and that is abnormal. As Tjebbes and Wriedt seem to suggest, it *may* have been due to crossing-over between the X- and the Y-chromosome in the yellow male—yellow being sex-linked. But that alone would be sufficient to explain the results, and the dominance of the black is superfluous (Doncaster, 1912

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[1] These breeding results have not yet been published. They will appear later when more facts have been collected.

and 1913, pp. 21 and 22; Bamber, 1927, pp. 19–23). If they mean to insist on the *dominance* of the black, as they evidently do, this implies that they have overlooked the fact that it is the presence of the *yellow* in their male kittens which is unexpected, *not* the presence of the black.

It will be interesting to see the results of further matings involving dominant black and yellow.

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THE HYPOTHESIS OF CHROMOSOME AFFINITY AND THE PHENOMENON OF SUPPRESSION OF CHARACTERS ON CROSSING.

By EDMUND MALINOWSKI.

SEVERAL papers have appeared recently which try to prove that some linkage phenomena may be caused by an association between particular chromosomes.

In the cytological literature we frequently find data concerning linkage of chromosomes. These linkage phenomena may be conditioned either by material connections between particular chromosomes, or by an unknown affinity which causes the chromosomes to show a tendency to go in groups to each pole of the dividing cell. S. Nawashin[16], R. R. Gates[5], Ö. Winge[23, 24], Z. Wóycicki[25], R. E. Cleland[2] and other authors point out the existence of material connections between the chromosomes of certain plants. F. Schrader[19] shows that in some *Pseudococcus* species there are two groups of chromosomes, and that "the perfectly definite distinguishing characteristics of each of the two chromosome groups make it possible to follow them through the entire division, and it is plain that the clumped group acts as a unit and that all its members go·to one and the same pole." Schrader does not, however, figure any material connections between the chromosomes of those groups.

Cleland[2] points out another kind of affinity which leads to the same results. According to him "every chromosome seems to have some sort of affinity for a particular pole, otherwise the picking out of alternate chromosomes to be sent to the same pole is inexplicable. Each chromosome must go to one pole rather than the other, and as this applies to every chromosome in the cell, it naturally follows that in every cell the same chromosomes will go together to the poles. The effect will be the same as though the chromosomes were actually bound together structurally in such a way as to form one pair of large compound chromosomes."

A. B. Stout[20] describes in *Carex* an interesting case of arrangement of chromosomes in series. This serial arrangement is not lost in the division stages. The daughter chromosomes are arranged in a series which remains in evidence during the resting stages.

R. R. Gates(5) believes that the coalescence of chromosomes, observed by him in *Lactuca,* may furnish a basis for the phenomena of linkage as distinct from those based upon relations between the two members of a given pair of chromosomes.

The idea as to the possibility of existence of chromosome coupling as a basis for linkage phenomena was suggested also by Rennet in 1917 ((17) footnote, p. 248).

In 1925 several papers were published in which we find genetical arguments for the possibility of the existence of such a higher order of linkage. I myself published four papers during that year(10–13) concerning this phenomenon in wheat, and the arguments given in those papers were repeated by me in vol. XVII of this *Journal*(14).

In the same year, 1925, O. Renner published a paper(18) in which he discusses the possibility of the existence of "Chromosomenkoppelung" in *Oenothera,* and brings forward the following arguments in favour of this supposition:

(1) If the linked factors (for instance the factors *M, N, P, S, R*) were located in one chromosome "das fragliche Chromosom müsste also sehr lang sein, wenn es drei oder noch mehr Genen die Möglichkeit geben sollte sich als selbständig zu gebärden."

(2) If the linked factors were located in one chromosome the mechanism of crossing-over would be different from that in *Drosophila* because in the cases observed by Renner "die Koppelung bald mehrfach, bald einmal, bald gar nicht gebrochen wird, je nach dem Grad der Aehnlichkeit des Partnerchromosoms."

Recently Hurst(8) has put forward some evidence for the existence of superlinkage in *Rosa.* The fundamental number of chromosomes in *Rosa* is 7. Somatic numbers are diploid 14, triploid 21, tetraploid 28, pentaploid 35, hexaploid 42, or octoploid 56. Hurst believes that "the significance of the septuple numbers of chromosomes in *Rosa* is apparent in various stages of gametogenesis and also in some somatic divisions in diploids and polyploids, in which it is evident that the chromosomes are working in sets of seven or septets." When comparing the taxonomic characters of the species in the living collection at Kew Hurst was struck by the fact that the tetraploid species showed the combined characters of two distinct diploid species, while the hexaploid species showed the combined characters of three distinct diploid species, and the octoploid species showed the combined characters of four distinct diploid species.

In mice W. H. Gates(6) found some characters which show an association apart from linkage phenomena in Morgan's sense; for there was

a tendency for the entire group of characters derived from each parent to associate together. This association appears only in specific crosses, and is believed by the author to be due to a grouping of the chromosomes.

Phenomena of this nature appear to be not uncommon, and the hypothesis of "superlinkage" has been supported by means of more or less numerous genetic arguments by several authors almost simultaneously.

It is probable that the affinity between certain chromosomes may be greater than between others. The existence of different degrees of affinity between particular chromosomes may furnish the basis for the phenomenon of exchange of elements between two sets of chromosomes of different origin. Cleland(2) believes that such a phenomenon exists in *Oenothera* and he calls it "chromosome exchange." If this really happens we should expect that in the F_2 generation from certain crosses one or both parental types would not appear at all. Possibly in this way we might explain some cases of suppression of characters on crossing, and so throw light upon the so-called "Wichura" type of segregation.

Suppose for instance that we have two types differing from one another in three cumulative factors, and that these factors are each located in a different chromosome. We have thus three chromosomes A, B, C in one parent and three respectively homologous chromosomes a, b, c in the other one. The genetic constitution of the first parent will be $AABBCC$ and of the second one $aabbcc$. Let us suppose further that two of these chromosomes, A and B, are linked with one another, and that the chromosomes a and b are also linked. The precise nature of the linkage does not matter. It may be structural, or there may exist a sort of chemical affinity between the chromosomes. If this affinity be greater between chromosomes A and b than between A and B, then in F_1 the normal reassortment of A and a with B and b will be upset and we shall obtain the new permanent combinations Ab and aB. If chromosomes C and c are independent of the others the F_1 hybrid will

	AbC	aBC	Abc	aBc
AbC	4	4	3	3
aBC	4	4	3	3
Abc	3	3	2	2
aBc	3	3	2	2

Diagram 1.

produce the following four kinds of gametes: *AbC*, *aBC*, *Abc*, *aBc*. In F_2 we shall obtain various intermediate types, as shown on Diagram 1, but the parental types, *AABBCC* and *aabbcc*, will not reappear. In the F_2 generation we shall obtain three kinds of individuals, according as they contain 2, 3, or 4 factors; but none will possess 0 or 6 factors, *i.e.* there will be no parental combinations in F_2. This is what Vavilov[21] speaks of as the Wichura type of segregation.

Wichura[21] worked with the hybrids of *Salix* species, and obtained in his crosses an intermediate F_1 generation without apparent segregation in F_2. From the photographs reproduced in Heribert-Nilsson's paper[7] it would seem that the parental types did not reappear in the F_2 generation from *Salix viminalis* × *S. caprea*. Individuals resembling *S. viminalis* did not appear at all. One single F_2 individual noted by Heribert-Nilsson as possessing "ganz caprea-ähnliche Blätter" cannot be identified with *S. caprea* in respect of the width of the leaves. Heribert-Nilsson made a back cross (*viminalis* × *caprea*) × *viminalis* and obtained in the subsequent generation "ganz viminalis ähnliche Blätter." But these "viminalis ähnliche Blätter" were not identical with the leaves of the *viminalis* parent in respect of width.

F. L. Engledow[4] has described the phenomenon of "shift" for the shape of the glumes in a *Triticum polonicum* × *Tr. durum* cross. F_1 was intermediate. "For F_2 the frequency-distribution of glume-length was very clearly trimodal, and its form suggested simple segregation on the 1 : 2 : 1 basis." Engledow examined a large material, making over 10,000 measurements of glume-length and following his investigations up to the F_3 generation. He found that the mean glume-length for *polonicum* is in F_2 "shifted" down by more than 20 per cent., that the shifted value breeds true, and is again exhibited by F_3 *polonicum* ex intermediate F_2 forms. For *durum* "a corresponding small upward shift seems to have occurred." Engledow writes ([4], p. 93) that "the parental *polonicum* does not reappear in F_2. In its place are found *polonicum* plants which closely resemble parental *polonicum* in general appearance, but whose mean glume-length is more than 20 per cent. lower than that of *P* (parental *polonicum*). This 'shifted' form, when selfed, breeds true."

There is a tendency among geneticists to believe that segregation phenomena like those in the *Salix* crosses may be explained by means of the multiple-factor hypothesis. Non-appearance of parental types in the F_2 generation is usually believed to be due to a very great number of factors conditioning the differences between these types. Engledow,

however, has given much evidence which favours the view that in the *polonicum* × *durum* crosses it is "impossible to devise a suitable multiple-factor explanation," or at least that any such explanation is a problematical one. He says that "'Multiplying factors' have been suggested by some in explanation of the phenomena but no feature of these results appeared to lend any encouragement to that idea" ((4), p. 81).

It is difficult also to explain a "shift" of the type of the apical tooth of *durum* wheat observed by Vavilov(21) in a *Triticum persicum* × *Tr. durum* cross by the hypothesis of cumulative factors. Vavilov reports that in the F_2 generation no plants similar to *Tr. durum* were observed, nor were any found reproducing exactly *Tr. persicum* in glumes and teeth. A detailed investigation of over 4000 F_3 plants revealed no individuals with glumes of the type of *Tr. durum*. "This cross," says Vavilov(20), "was perfectly fertile and there could be no missing series of forms as it happens with distant crosses." Vavilov concludes that the explanation of this case by means of the multiple-factor hypothesis "is practicable if we admit a very great number of factors conditioning the shape of the glumes." But, as he says later on, "it is possible that in this case the scheme of cumulative factors hypothesis can not be applied at all."

If we assume that in crosses with a Wichura type of segregation the phenomena of chromosome exchange[1] occur with the production of new permanent chromosome compounds, then with such restrictions the multiple factor hypothesis will be applicable to this type of segregation.

In the *Triticum polonicum* × *Tr. durum* cross it is possible that only two pairs of allelomorphs are involved.

Let us suppose that the glume-length of *polonicum* is determined by two factors A and B, one of which, A, produces the essential glume-length characteristic of *Tr. polonicum*, while the second, B, increases this essential length by more than 20 per cent. The allelomorph of A will be a, which determines the length of *durum* glumes. The heterozygate Aa is intermediate. The factor B, which increases the glume-length of *polonicum* by more than 20 per cent., is scarcely appreciable on the *durum* type[2].

The factors A and B are located in different chromosomes which are linked with one another. The chromosomes a and b of *Tr. durum* are

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[1] I suggested this idea in one of my former papers (15).

[2] A certain analogy exists between the factor B and the factor increasing the glume-length which I observed in my *polonicum* × *dicoccum* crosses (14). But the factor increasing the glume-length in my experiments exerted a much more pronounced influence upon the *polonicum* glumes than upon the glumes of *dicoccum*. This factor, however, did not show any linkage with other factors.

also linked. In F_1 new permanent combinations will occur, viz. Ab and aB, and the F_1 individual will produce only two kinds of gametes, viz. Ab and aB. In F_2 we shall get three types of individuals, namely: $AAbb$, $AaBb$, $aaBB$ in the proportion of $1:2:1$. The type $AAbb$ will be *polonicum* "shifted" down by more than 20 per cent., the type $aaBB$ will be *durum* with slightly increased glume-length and the type $AaBb$ will be intermediate like F_1. This type, according to Engledow's statement, "remains singularly constant from generation to generation."

In the case of Engledow's experiments the formation of a new permanent chromosome association is rendered more probable owing to linkage between several characters. Engledow points out that in the "shifted" *polonicum* "grain-length exhibited shift on lines precisely corresponding to the case of glume-length and a group of ten characters —five of the glume and five of the grain—behaved as 'genetic inseparables'" ((4), p. 81). It is possible therefore that the chromosome carrying the factor B contained also some other factors influencing grain and glume characters, and that all these factors were conveyed together from one chromosome group to another.

Some cases of the Wichura type of segregation are known in which one of the parental types undoubtedly appears in the F_2 generation whilst another one does not appear at all. Such a case was observed by E. M. East(3) in *Nicotiana Langsdorfii* × *N. alata* crosses in relation to flower shapes. East writes as follows: "Individuals reproducing the *N. Langsdorfii* were found in the F_2 generation....Certain of these F_2 individuals reproduced *N. Langsdorfii* populations in the F_3 generation.... No F_2 individuals reproducing *N. alata* were found, but F_3 plants approaching such a type were produced." The F_1 generation was intermediate, nearer however to *N. Langsdorfii*. East concludes as follows: "Though extremes like each parent were not produced, it is hardly possible to see any other cause for this great difference in variability than segregation and recombination of Mendelian factors." But East does not propose any definite Mendelian scheme of segregation, and Engledow(4) believes that the multiple-factor action "is perhaps...a matter of uncertainty...even in such careful investigations as those of East."

Possibly such cases may be explained by means of cumulative factors which in the heterozygous state produce almost the same effect as in the homozygous one.

I found some evidence for the existence of such factors in my crosses between varieties of *Phaseolus vulgaris*(9). In the crosses Hinrich's

Riesen × Bagnolet and Flageolet rouge × Bagnolet I found that the seeds of F_1 are as long as the seeds of Bagnolet, which was the largest parent in regard to the shapes of seeds. In F_2 a segregation occurred; both parental types appeared but the Mode of the polygon of variation of seed-length was the same in F_2 as in Bagnolet. I suggested that there are several cumulative factors increasing the basal seed-length. I assumed this basal length to be approximately 10 mm., and that each factor increased this basal length by approximately 2 mm. I assumed also that each of those factors in the heterozygous state increased the length of the seeds by the same or almost the same amount.

The case observed by East in *Nicotiana* might possibly be explained in the same way, with, however, one essential difference, viz. the occurrence of a "chromosome exchange."

Let us suppose that in a case of seed-length we have three cumulative factors A, B, C, each located in a different chromosome, and that A and B are linked. Suppose the basal length to be 10 units, and that each factor increases it by 2 units, the seed-length being 16 units in one of the parental types and 10 in the other. The genetical constitution of one parent will be $AABBCC$ (chromosomes A and B are linked) and of the second one—$aabbcc$ (chromosomes a and b are linked). If the supposed affinity between the chromosomes A and B is less than between A and b, then in F_1 we shall obtain new permanent combinations Ab and aB instead of AB and ab. The third chromosome being independent we shall get the following kinds of gametes: AbC, aBC, Abc, aBc. In F_2 we shall obtain three kinds of individuals, some of which will contain two factors, and others three or four factors. But the parental combinations will not occur (Diagram 2).

	AbC	aBC	Abc	aBc
AbC	4	6	4	6
aBC	6	4	6	4
Abc	4	6	2	4
aBc	6	4	4	2

Diagram 2.

Since we are assuming that each factor here produces as great an effect in the heterozygous state as in the homozygous one we should in such a case obtain only three classes of individuals as judged by their

external appearance, viz. showing 16 units of length (10 + 6), or 14 units (10 + 4) or 12 units (10 + 2). The larger parental type would appear in F_2 but not the smaller.

This scheme may possibly be applied to the case of *Nicotiana*. We must assume, however, that instead of dominant factors increasing the shapes there are dominant factors decreasing the shapes; and we must further assume a larger number of factors.

Perhaps, too, the hypothesis of chromosome affinity will help to elucidate some aberrant cases of Mendelian ratios to which Bateson called attention in his paper on Segregation(1); and we may now examine two theoretical possibilities resulting from "chromosome exchange," without, however, definitely relating them to concrete cases of "aberrant inheritance."

Let us suppose that two factors A and B determine red colour. These two factors are located in different chromosomes which are linked with one another. After crossing a red (AB) individual with a white one (ab), we shall obtain an F_1 of the genetic constitution $AaBb$. The F_1 individuals will be red because A and B are dominant factors. If the affinity between the chromosomes A and b is greater than between A and B, then we shall obtain in F_1 two kinds of gametes, namely Ab and aB and in F_2—the combinations $BBaa$ (white): $BbAa$ (red): $bbAA$ (white) in the proportion of 1 : 2 : 1. Thus the number of white individuals will be the same as that of red individuals and, in such a way, instead of expected ratio 3 red : 1 white, we shall obtain the ratio 1 red : 1 white.

Let us suppose now that A and B are not dominant factors and that the F_1 generation is intermediate in respect of colour. Then in the F_2 generation instead of red and white individuals in the ratio of 1 : 1 we shall obtain intermediate and white individuals in the ratio of 1 : 1. In the last case red individuals will not appear at all in the F_2 generation.

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SEX-RATIO IN PHEASANT SPECIES-CROSSES.

By ROSE HAIG THOMAS and JULIAN S. HUXLEY.

(1) Introduction.

The crosses recorded in the following pages were made in the twelve years 1906–1918, by one of us (R. H. T.), who is thus entirely responsible for the data, while the other (J. S. H.) is responsible for the whole theoretical analysis. The work encountered great difficulties owing to war restrictions, and eventually had to be abandoned. However, a number of points of interest were established, which it is the purpose of the present paper to present and analyse. The two most important concern the remarkable abnormalities of sex-ratio, and the degree of Mendelian segregation and recombination occurring. The latter point will be dealt with in a later paper. The figures are totals and thus include and supersede those previously published (Smith and Haig Thomas, 1913).

(2) Crosses made. Sterility and Fertility.

Crosses were attempted between the following 13 species belonging to 7 genera of game birds, mostly pheasants:

Phasianus colchicus	*Thaumalea amhersti*
P. formosanus	*T. picta*
P. reevesi	*Lophophorus impeyanus*
P. versicolor	*Ceriornis caboti*
Gennaeus nycthemerus	*Numida mitrata*
G. swinhoei	*Gallus sonneratus*
G. praelaetus	

Some of the crosses were invariably sterile—*i.e.* no eggs were produced; others were invariably infertile—*i.e.* eggs were laid, but none ever hatched, owing apparently to the absence of fertilisation; while others were fertile. Great variations, as was to be expected, were found in the fecundity (number of eggs produced) of the infertile and fertile classes; in the fertility (proportion of dead in shell *plus* hatched young) of the fertile class; and in the embryonic viability ("hatchability") of the fertile class. In addition, certain types of crosses were in some

individual cases sterile, in others fecund, other types were in some cases infertile, in other cases fertile. This has been indicated in Tables I and II.

TABLE I.

(♀ parent first; number of individuals used in brackets.)

I. Sterile Crosses.

(*a*) wholly sterile in all cases; (*γ*) fecund elsewhere; (*γ₁*) fecund but sterile elsewhere; (*γ₂*) fertile elsewhere.

1st Crosses:

(*a*)   T. amhersti (2) × L. impeyanus (1)
        N. mitrata (1) ⎫ × C. caboti (1)
        T. amhersti (1) ⎭
        P. versicolor (1) × (G. nycthemerus × swinhoei) (1)
        C. caboti (1) × P. formosanus (1)
        P. colchicus (several) × (P. reevesi × versicolor) (3)

(*γ₁*) P. versicolor (1) × P. reevesi (1)

(*γ₂*) G. swinhoei (1) × G. nycthemerus (1) (but very poor fecundity)

Back-crosses:

(*a*)   (P. reevesi × versicolor) (1) × P. versicolor (1)
        (P. reevesi × formosanus) (1) × P. formosanus (1)

(*γ₂*) P. formosanus (1) × (P. formosanus × versicolor) (1)
            (This hybrid elsewhere fertile in both sexes.)

Inter se Crosses:

(*a*)   (P. reevesi × versicolor) (2) × (P. reevesi × versicolor) (4)
        (P. reevesi × colchicus) (1) × (P. reevesi × colchicus) (1)
        {T. picta × [amhersti × (amhersti × picta)]} (4) × brothers (2)
        [(P. amhersti × picta) × picta] (8) × [(P. amhersti × picta) × picta] (9)

(*γ₁*) (P. reevesi × formosanus) (2) × (P. reevesi × formosanus) (1)

(*γ₁* and *γ₂*) (P. formosanus × versicolor) (1) × (P. formosanus × versicolor) (1)
        {G. nycthemerus × [nycthemerus × (nycthemerus × swinhoei)]} (1)
                                                        × brother (1)

(*γ₂*) (G. nycthemerus × swinhoei) × (G. nycthemerus × swinhoei) (1)

(*γ₁*) (T. amhersti × picta) (6) × (T. amhersti × picta) (6)

(*γ₁* and *γ₂*) (T. picta × amhersti) (1) × (T. picta × amhersti) (1)

(*γ₂*) (T. picta × amhersti) (1) × (T. amhersti × picta) (1) (poor fecundity elsewhere)

II. Infertile Crosses.

(A) wholly infertile; (B) wholly infertile, but fertile in the reciprocal cross;
(C) same hybrid elsewhere fertile in other similar crosses.

		No. of eggs produced
1st Crosses:		
(A)	Gennaeus nycthemerus (2) × Gallus sonneratus (1)	40
	P. reevesi (2) × L. impeyanus (1)	27
	C. caboti (1) × L. impeyanus (1)	2
	{[(G. swinhoei × nycthemerus) × swinhoei]} (1) × G. praelactus (1)	5
(B)	P. versicolor (4) × P. reevesi (2)	48
(C)	P. reevesi (2) × P. versicolor	16
	P. reevesi (1) × P. colchicus (1)	11
	P. versicolor (1) × P. formosanus (1)	1
	G. swinhoei (2) × P. reevesi (2)	19

TABLE I (*continued*).

	No. of eggs produced
Back-crosses:	
(A) P. reevesi (3) × (G. swinhoei × P. reevesi) (2)	128
[(P. formosanus × versicolor) (1) × P. versicolor (1)	1
(P. versicolor × formosanus) (4) × P. formosanus (2)	10
(P. formosanus × versicolor) ♀ is fertile	
T. amhersti (3) × [T. amhersti × (amhersti × picta)] (1)	69
Reciprocal not made. ♂ and ♀ of same constitution elsewhere fertile.	
(B) (T. amhersti × picta) (7) × T. amhersti (2)	170
(C) (P. formosanus × versicolor) (1) × P. versicolor (1)	2
(G. swinhoei × nycthemerus) (1) × G. nycthemerus (1)	14
Inter se Crosses:	
(A) (P. reevesi × formosanus) (1) × (P. reevesi × formosanus) (1)	4
(P. versicolor × formosanus) (3) × (P. versicolor × formosanus) (3)	18
(F₂ from (P. formosanus × versicolor) is fertile)	
(C) (P. formosanus × versicolor) (1) × (P. formosanus × versicolor) (1)	9
{G. nycthemerus × [nyc. × (nyc. × swinhoei)]} (2) × brothers (2)	33
(T. picta × T. amhersti) (2) × (T. picta × T. amhersti) (2)	17
(T. amhersti × T. picta) (9) × (T. amhersti × T. picta) (9)	184
(T. picta × amhersti) × (T. amhersti × picta)	1

With regard to these points, the results may be briefly summarised as follows:

(1) None of the inter-generic crosses involving *Lophophorus, Ceriornis, Numida* or *Gallus* were fertile. Within the three genera *Gennaeus, Phasianus* and *Thaumalea*, only two inter-generic crosses were attempted ($G \times P$ and $P \times T$), both were successful, in one case each. Another involving *Phasianus* and a F_1 *Gennaeus* species-hybrid was sterile. The two successful crosses only gave five hatched young in all, and of these five, only one, a male, was raised to maturity. This when back-crossed to a female of the pure paternal species proved wholly infertile.

(2) The fertile intra-generic crosses may be divided into three sub-classes—(i) where the species crossed are only distantly related, (ii) where they are moderately closely related, (iii) where they are closely related. The first two groups gave 20 to 25 per cent. of hatched young, the last about 40 per cent. All the six attempted combinations of *Phasianus* species were fertile except one, which, however, was fertile in the reciprocal cross. The two reciprocal crosses between the two species of *Gennaeus* were both fertile, but the fecundity was much better when *G. nycthemerus* was used as female parent. The two (closely-related) species of *Thaumalea* were abundantly fertile in both combinations. Numerous back-crosses were made, and were mostly fertile. As was to be expected, a greater proportion of the F_2 crosses than of the back-crosses were sterile, although the percentage of fertility in the fertile crosses of the two types did not differ apparently.

TABLE II.

Cross No.	Fertile crosses (♀ parent first)	Total No. eggs	In-fertile	Hatched	Dead in shell ♀	♂	?	Dead immature ♀	♂	?	Reared to maturity ♀	♂	Total sexed ♀	♂	% ♂♂ Of total sexed	Of dead imma-ture	Of dead in shell	% of total in-fertile	% of fertile dead in shell	% of fertile dead imma-ture	% of hatched imma-ture	% of total hatched dead
	I. First Crosses:																					
	(A. Inter-generic):																					
1	G. swinhoei × P. reevesi	?	?	4	?	?	?	—	—	3	—	1	—	1	(100)	(0)	—	?	?	?	75.0	?
2	P. colchicus × T. picta	16	14	1	—	—	1	1	—	—	—	—	1	—	(0)	(0)	—	87.5	50.0	50.0	100.0	6.25
	(B. Intra-generic)																					
	(B 1: distant):																					
3	P. reevesi × P. versicolor	63	47	13	—	—	3	1	4	—	3	5	4	9	69.2	80.0	—	74.6	18.8	31.3	38.5	20.7
4	„ × P. formosanus	36	?	8	?	?	?	—	4	—	1	3	1	7	87.5	100.0	—	?	?	?	50.0	22.2
5	„ × P. colchicus	30	20	7	—	—	3	1	—	—	1	4	2	4	66.7	(0)	—	66.7	33.3	100	14.1	23.8
	All B 1												(7)	(20)	(74.1)	(80.0)						
	(B 2: intermediate):																					
6	P. versicolor × P. formosanus	103	90	11	1	1	—	—	4	—	4	3	5	8	61.5	100.0	50.0	87.4	15.4	30.8	36.4	10.7
7	P. formosanus × P. versicolor	44	—	5	—	—	—	—	—	—	2	3	2	3	60.0	—	—	—	—	—	0	11.4
8	G. nycthemerus × G. swinhoei	27	—	20	—	—	—	—	—	1	7	12	7	12	63.2	—	—	0	—	—	5.0	14.1
9	G. swinhoei × G. nycthemerus	2	—	1	1	—	—	—	—	—	1	—	2	—	0	—	0	—	50.0	0	0	50.0
	Total B 2												(16)	(23)	(59.0)	(100.0)	(33.3)					
	(B 3: close):																					
10	T. amhersti × T. picta	120	—	41	—	—	—	1	10	6	11	13	12	23	65.7	90.9	—	—	—	—	41.5	34.2
11	T. picta × T. amhersti	100	—	50	—	—	—	5	14	—	16	15	21	29	58.0	73.7	—	—	—	—	38.0	50.0
	Total of B 3												(33)	(52)	(61.2)	(80.0)						
	Total of First Crosses	541	—	161	(2)	(1)	(7)	9	36	10	46	59	57	96	62.7	80.0	(33.3)	—	—	—	—	—
	II. Back-crosses:																					
	(from I, B 2):																					
12	(G. nyc. × G. swin.) × G. swin.	62	51	9	—	2	—	—	—	4	1	4	1	6	85.7	—	100.0	82.3	18.2	36.4	41.4	14.5
13	G. nyc. × (G. nyc. × G. swin.)	42	—	11	—	—	—	—	—	4	2	5	2	5	71.4	—	—	—	—	—	36.4	26.2
14	(G. swin. × G. nyc.) × G. nyc.	12	9	1	1	1	—	1	—	—	—	—	2	1	33.3	0	50.0	75.0	66.7	33.3	100.0	8.3
15	[G. nyc. × (G. nyc. × G. swin.)] × G. nyc.	45	—	9	—	—	—	4	3	—	2	—	6	3	33.3	42.9	—	—	—	—	77.8	20.0
16	G. swin. × [[G. nyc. × G. swin.] × G. swin.] × G. nyc.	24	—	5	—	—	—	1	1	—	2	1	3	2	40.0	50.0	—	—	—	—	40.0	20.8

Table III (continued): Back-crosses and Hybrids inter se

No.	Cross																					
17	G. nyc. × [G. nyc. × (G. nyc. × G. swin.)]	53	—	25	—	—	4	10	—	3	8	7	18	72·0	71·4	—	—	—	—	—	56·0	47·2
18	[(G. nyc. × G. swin.) × G. swin.] × G. swin.	4	3	1	—	—	—	—	—	—	1	—	1	100·0	—	—	75·0	—	0	0	0	25·0
19	(P. form. × P. vers.) × P. vers. (from I, B 3):	48	—	16	—	—	—	9	—	5	2	5	11	68·8	100·0	100·0	—	—	—	—	56·3	33·3
20	(T. amh. × T. picta) × T. amh.	170	170	—	—	—	—	—	1	5	6	5	6	54·5	—	—	100	—	—	—	—	0
21	T. amh. × (T. amh. × T. picta)	68	—	12	10	1	5	24	1	6	6	5	41	80·4	82·7	83·3	12·1	22·4	60·4	8·3	8·3	7·6
22	(T. amh. × T. picta) × T. picta	66	8	45	2	—	1	3	6	7	7	10	5	50·0	75·0	100·0	64·7	16·7	33·3	77·8	77·8	68·2
23	(T. picta × T. amh.) × T. amh.	34	22	10	—	—	3	3	2	—	—	5	5	38·5	50·0	—	—	—	33·3	40·0	40·0	29·4
24	[T. amh × ((T. amh. × T, picta)] × T. picta	54	—	19	—	—	—	3	6	2	2	8	5	—	—	—	—	—	—	63·2	63·2	35·2
25	T. picta × [T. amh. × (T. amh. × T. picta)]	30	—	11	—	—	—	2	—	4	5	4	7	63·6	100·0	83·3	—	—	—	18·2	18·2	36·7
	Total Back-crosses	712	—	174	3	15	19	55	23	36	41	58	111	65·7	74·3	83·3	—	—	—	55·7	55·7	24·4
	III. HYBRIDS inter se: (from I, B 2):																					
25a	(P. vers. × P. form.) × (P. form.)	24	23	0	—	1	5	—	—	—	5	10	—	—	—	—	—	—	—	—	—	—
26	(P. form. × P. vers.) × (P. form. × P. vers.)	171	104	53	—	10	5	36	2	5	5	10	45	81·8	87·8	100·0	60·8	29·9	64·2	81·1	81·1	31·0
27	(G. nyc. × G. swin.) × (G. nyc. × G. swin.)	158	79	71	3	—	17	24	8	3	9	23	38	62·3	58·5	62·5	50·0	10·1	62·0	69·0	69·0	44·9
28	[(G. swin. × G. nyc.) × G. swin.] × (G. nyc × G. swin.)	10	—	5	—	1	1	1	—	2	1	3	2	40·0	50·0	—	47·6	13·6	—	40·0	40·0	50·0
29	{G. nyc. × [nyc. × (nyc. × swin.)]} × brother	42	20	19	—	3	—	6	3	5	5	5	11	68·8	75·6	51·4	50·1	14·4	40·9	47·4	47·4	40·5
30	(T. amh. × picta) × (T. amh. × picta)	770	386	329	17	20	48	149	18	21	26	86	193	69·2	84·6	51·4	67·9	7·7	54·0	65·3	65·3	42·7
31	(T. picta × amh.) × (T. picta × amh.)	81	55	24	1	—	2	11	—	6	5	9	16	64·0	84·6	(0)	16·7	7·7	50·0	54·2	54·2	29·6
32	(T. picta × amh.) × (T. amh. × picta)	6	1	1	—	4	—	—	1	—	—	—	—	—	—	—	16·7	80·0	20·0	100·0	100·0	16·7
33	[(T. amh. × picta) × amh.] × brother	15	9	3	—	3	1	1	—	—	1	1	2	(66·7)	(100·0)	(50·0)	60·0	50·0	33·3	66·7	66·7	20·0
	Total inter se crosses	1277	—	505	21	42	74	228	32	42	52	137	307	69·1	75·5	56·3	—	—	—	66·1	66·1	39·5
	Grand total:	2530	—	840	26	50	102	319	65	124	152	252	514	67·1	75·8	62·3	—	—	—	57·9	57·9	33·2

Brace totals (Grand total): 119, 486, 276, 766

We may now turn to a consideration of the sex-ratios. Details are given in Table II. The first point that strikes the eye is the almost invariable predominance of males. On the total from all offspring of species-crosses, the sex-ratio is 67·1 per cent. male on a total of 766 birds —a ratio of 204 male : 100 female.

This preponderance of males is equally marked in the separate groups. The percentage of ♂♂ is 62·7 in the offspring of first crosses, 65·7 in the offspring of back-crosses, and 69·1 in the offspring of F_2 and later *inter se* crosses.

This is an excellent illustration of Haldane's Rule (Haldane, 1922), viz. that where, in the offspring of a species or varietal cross, the numbers of one sex are deficient, the deficiency is in the heterogametic sex—in mammals for instance the males, in birds and Lepidoptera, the females.

The excess of males is to be seen in the great majority of the individual matings. There are excesses of females only in two cases among broods where more than five individuals were sexed. The excess of males also occurs equally in the F_1 offspring of distant, intermediate, and close intra-generic crosses. As only two individuals from inter-generic crosses were sexed, no conclusions can be drawn from these.

The next step is to analyse the sex-ratios further, using the age at death as criterion. From the records, it is possible to divide the sexed birds into three classes: (1) those which reached maturity; (2) those which hatched but died immature; (3) those which died in shell. Those of classes (2) and (3) were (in the years 1917 and 1918) not always sexed, owing to difficulties during the war; but sufficient numbers, especially of (2), are available. The great majority of class (2) died at under 6 weeks of age, and from natural causes: accidental death accounts for very few.

If we take the figures for the whole lot, we find that the ♂ percentage of the dead-in-shell was 62·3, of the dead immature 75·8, and of those raised to maturity only 55·1. Owing to war conditions, 22 ♀ and 51 ♂ of the F_2 from the *T. amhersti* × *T. picta* cross were killed when young; these have been included in class (2). But even if we deduct these, the percentage for class (2) remains very similar, 77·0 instead of 75·8 (the rise would be expected, since if usually more males die young than reach maturity, these killed young should include those with expectation of reaching maturity and therefore lower sex-ratio).

The same marked difference between the sex-ratio of classes (1) and (2) is seen in every type of mating, that of class (2) (in all lots of 10 or over) being from 74 to 80 per cent.; of class (1) from 51 to 56, with

one exception of 74 per cent. There are many less individuals in class (3), and the figures are not concordant. In the hybrids *inter se* the ♂ ratio of dead-in-shell is only 56·3, in the back-crosses it is 83·3 (but on only 18 specimens). The class (3) (dead-in-shell) average for all matings, on 69 specimens, has a ♂ ratio higher by 7·2 per cent. than that of those raised to maturity.

Leaving aside the differences between classes (2) and (3), it is abundantly clear that the mortality of males is much greater than that of females in the early period of life.

If we take the figures after deducting those killed young, the ♂ percentage for all sexed is 67·8, while that for those dying in shell or immature is 74·6. That for those hatched dying immature is 77·0.

The higher earlier ♂ mortality in itself makes it very improbable that the abnormally high ♂ ratio can be due to differential mortality; further, as pointed out earlier (Smith and Haig Thomas, 1913) in some cases such a high proportion of all eggs laid were raised that differential mortality is here definitely excluded.

It is difficult to give any but very hypothetical explanations of these facts. I would suggest that very possibly the high male ratio in these crosses is due to a true reversal of sex. In Goldschmidt's *Lymantria* experiments (full references in Goldschmidt, 1927), either intersexual females or males (sex-reversed females) were produced in place of normal females in the F_1 of all matings of a certain type. If a similar process occurred in forms like birds, with endocrine control of sex, it would be expected that once the critical point was past and the type of development switched over from female to male, the endocrine control would ensure that all such birds would appear male.

In Goldschmidt's work, however, the abnormality only occurred when males of "strong" races were mated with females of "weak" races, and not in reciprocal crosses. We may attempt to see if anything of the sort is visible here. Unfortunately the F_1 numbers are not large, but it seems clear that *T. amhersti* × *T. picta* gives more ♂♂ than the reciprocal (65·7 as against 58·0 per cent.); as does *G. nycthemerus* × *G. swinhoei* than its reciprocal, though the numbers here are lamentably small. On analogy with Goldschmidt's work, we may call *amhersti* and *nycthemerus* "weak" races (with slow-acting sex-determiners) and *picta* and *swinhoei* "strong" races (with rapidly-acting sex-determiners). The *Phasianus* F_1 numbers are too small to give any indication, but for reasons which will be apparent later, it is probable that *formosanus* (F) is "weak" and *versicolor* (V) "strong." If we make these assumptions, then

we can by analogy with *Lymantria* make certain prophecies as to sex-ratio. Let us call the assumed "weak" species W, the assumed "strong" forms S.

(I)   The following crosses should give females in defect.

      (A) *Gennaeus*   (B) *Thaumalea*   (C) *Phasianus*

    (W = nyc., S = swin.)   (W = amh., S = picta)

Cross Nos. :

        8: $W \times S$ 10: $W \times S$ 7: $F \times V$

     12: $(W \times S) \times S$ 21: $W \times (W \times S)$ 19: $(F \times V) \times V$

     13: $W \times (W \times S)$ 22: $(W \times S) \times S$ 26: $(F \times V) \times (F \times V)$

     17: $W \times [W \times (W \times S)]$ 24: $[W \times (W \times S)] \times S$

     27: $(W \times S) \times (W \times S)$ 30: $(W \times S) \times (W \times S)$

     (29: $W \times [W \times (W \times S)]$)

(II)   In the following crosses, ♀ defect is not expected.

      (A) *Gennaeus*   (B) *Thaumalea*   (C) *Phasianus*

Cross Nos. :

        9: $S \times W$ 11: $S \times W$ 6: $V \times F$

     14: $(S \times W) \times W$ 23: $(S \times W) \times W$

     16: $S \times [(W \times S) \times S]$ 25: $S \times [W \times (W \times S)]$

     15: $[W \times (W \times S)] \times W$ 31: $(S \times W) \times (S \times W)$

     (28: $[(S \times W) \times S] \times (W \times S)$) (I omit No. 33, which is complex, and only gave three sexed young)

The total figures for these classes are as follows (Table III):

TABLE III.

(I, ♀ defect expected; II, ♀ defect not expected. A, Gennaeus; B, Thaumalea; C, Phasianus.)

	Total hatched	Total sexed			Dead immature				Ratio of sexed dead immature as percentage of total sexed	Ratio of all dead immature as percentage of total hatched
		♀	♂	♂ ratio %	♀	♂	?	♂ ratio %		
I A	155	45	90	66·7	21	40	20	—	45·2	52·3
B	446	121	268	68·9	57	186	37	—	62·5	62·8
C	74	17	59	77·6	5	45	2	—	65·8	70·3
Total I	675	183	417	69·5	83	271	59	76·6	51·6	51·3
II A	21	16	8	33·3	7	5	—	—	50·0	57·2
B	95	39	57	59·4	8	30	2	—	39·6	42·1
C	11	5	8	61·5	—	4	—	—	30·8	36·4
Total II	127	60	73	54·9	15	39	2	72·2	44·1	40·4

The results are not materially altered if we omit all *Phasianus* crosses, and also crosses 28 and 29.

From this analysis, the following interesting facts appear. First, where defect of females is, on our premises, theoretically to be expected, the ♂ sex-ratios are not only far above 50 per cent., but also above those for the totals. Secondly, the ♂ sex-ratios *are* lower wherever excess

of ♀♀ is not theoretically expected. In one case they are well below 50 per cent. (It appears also that the higher the ♂ ratio where ♀ defect is expected, the higher it is also where ♀ defect is not expected.)

It seems clear therefore that there must be some resemblance between these species-crosses and those of Goldschmidt's in regard to the existence of "strong" and "weak" sex-determining factors, and that part at least of the abnormality of sex-ratio produced by species-crossing in pheasants is due to the coexistence of a single strong male-determining factor (X-borne) with a weak (if Y-borne) or intermediate (if autosomal) female-determining factor or factors whenever a cross of the type (weak ♀ × strong ♂) is made. On the other hand, it seems probable that this will not account for all the facts, especially the difference in ratio between *Gennaeus* and *Thaumalea* in the classes where ♀ defect is not expected.

We may now return to the question of differential mortality. We have just concluded that certainly a large proportion of the ♀ defect is to be ascribed to birds of ♀ chromosome-constitution becoming switched over to maleness, as is to be expected by analogy with *Lymantria*. If so, then these birds actually began existence as females and then the male-determining factor gained the upper hand, with consequent production of male hormone and remodelling of sex-organisation. It is not unreasonable to suppose that this would be a handicap to them in their development, and that therefore they would die more readily than other birds. If, as is very probable, the switch-over did not occur till near or after the end of embryonic life, the birds would be more sensitive when immature than as embryos. (In addition, if switch-over occurred comparatively late, the proportion of the sexes in embryonic life would be nearer equality, and we should therefore not expect, on purely statistical grounds, the ♂ mortality ratio to be so high for the embryonic period as later.) We might therefore expect a greater mortality among the immatures in the crosses where ♀ defect is expected. This appears to be the case, if we look at the last two columns of Table III. It will be seen that the proportion of those which died immature, whether measured as the ratio of sexed immature-dying birds to total sexed, or of all immature-dying birds to total hatched, is considerably higher in the crosses where ♀ defect is expected. That this difference is significant, however, we do not like to assert: many other factors might have been responsible as well. On the other hand, we should expect this state of affairs to be brought about by a marked decrease in the ♂ ratio of immature-dying birds in the classes where ♀ defect is not expected.

This, however, does not seem to be borne out by the facts (see ♂ sex-ratios for *total sexed* and *dead immature* in Table III). It is true that the figures for the class II are small, and also that the ratio here is below that of the class I; but the result is certainly not concordant with our expectation. It is worth while, however, to thresh out possibilities so that future workers may attempt an analysis on the accurate lines of Goldschmidt's experiments.

It is certain that no early ♂ mortality ratios approaching what are found here are obtained in ordinary Mendelian and varietal crosses in birds. In fowls, Jull (1924) found excess ♀ mortality before hatching; Crew (unpublished data kindly supplied to us), slight excess ♂ mortality before hatching. It is also important to note that one of us (R. H. T.) noted in sexing the birds that the gonads were often abnormal in size and form, which may well indicate that sex-reversal was occurring. Unfortunately these gonads were not preserved and only rough sketches were made at the time.

It can, however, be stated that late-hatching or weakly-hatching chicks and those dying at less than two days of age very frequently showed these abnormal gonads. Further, in a considerable number of cases a single small ovary was found associated with paired male gonads and ducts, the testes in such cases often being abnormally long. Here again it is unfortunate that war conditions prevented the histological examination of these gonads, but all the facts consistently point to a form of intersexuality which is likely to have represented a stage in sex-reversal.

Some of the F_2 and F_3 males from the *nycthemerus* × *swinhoei* series did not fight when penned together, which is invariable with normal males in such conditions. Possibly these represented incompletely reversed females.

Some extracts from notes made at the time are appended. All refer to chicks from *Phasianus* or *Gennaeus* crosses.

(*a*) Just hatched bird, killed for examination: normal ovary and oviduct; testes present, but abnormally large.

(*b*) Died at hatching: normal ovary and oviduct and vasa deferentia; testes a single large mass.

(*c*) Died at two days: small ovary, no oviduct; normal vasa, testes fused into a single irregular mass.

(*d*) Died young: on left, ovary and oviduct, on right a vas deferens.

(*e*) Died at two days: very similar to (*c*).

(f) Died young: ovary and oviduct on left side; two vasa deferentia, both on right side; no testes noted.

What does emerge clearly is that a marked upset of sex-ratio occurs in these species-crosses, usually in the direction of increased ♂ ratio. It is also highly probable that lack of balance between strong (rapidly-acting) and weak (slowly-acting) sex factors is at least an important cause in effecting this result. The fact of greater ♂ mortality in the immature stages is also perfectly definite, but the reasons for this are less clear, though we think there must be some connection between increased ♂ mortality and sex-reversal.

The phenomenon of high male sex-ratio in bird species-crosses is known in several other cases. It definitely occurs in pigeons, pheasants and grouse. In no case in birds where adequate numbers have been reared is an excess of females found. Haldane (1922) lays down a general rule applicable to all groups that "when in the F_1 offspring of a cross between two animal species or races one sex is absent, rare, or sterile, that sex is always the heterozygous [heterogametic] sex." As a matter of fact, reflection will at once show that this rule is a special case of the phenomena to be expected on the general principles of sex-determination advanced by Goldschmidt. On these principles, we should actually expect that if absence, deficiency, sterility (or other sex-abnormality) of one sex occurs after crossing, it should be manifested as follows: (A) in F_1: (i) in the heterogametic sex in one cross, (ii) in neither sex in the reciprocal cross; (B) in F_2: (i) in F_2 from cross (A) i, in the heterogametic sex, (ii) in F_2 from cross (A) ii, in the homogametic sex.

In some cases, e.g. Phillips (1916) no excess of males was found, and both sexes of F_1 males possessed testes, while the F_1 females were wholly without ovaries, possessing only a rudimentary oviduct. In addition, while the F_1 females of both reciprocal crosses were without gonads (a puzzling fact), those from one cross were henny-feathered, while those from the other showed marked approximation to cocky plumage. This may very likely represent incipient sex-transformation of the females; and presumably it is this cross which would represent "weak" ♀ × "strong" ♂ in Goldschmidt's terminology. Poll (1921), whose paper is not cited by Haldane, gives some further figures. He generally finds excess of males from hybridisation in pheasants and other gallinaceous birds, but little or none from hybridisation in ducks or finches. Suchetet (reference in Haldane, 1922), on the other hand, cites high male ratios in duck species-crosses; but Phillips (1915, 1921) agrees in general with Poll. In answer to queries, he writes as follows, kindly

giving information additional to that contained in his paper. He states that, as attention was mainly focussed on the males, unfortunately some females which died young may not have been recorded. The actual figures for four crosses (Mallard ♀♀ × Black Duck, × Pintail, × Florida Duck, × Australian Duck ♂♂) were:

	♂	♀
F_1	80	76
F_2	116	83
Back-crosses from F_1	95	95

The only at all exceptional ratio is that of the F_2, which gives a male percentage of 58. This is not nearly as large as that obtaining in the pheasant crosses, and must, for the reasons stated above, be regarded as probably in excess of the real male percentage. The figures are for adult or nearly adult birds (see below for discussion of this point).

He also kindly provided me with sex-ratio data on his various pheasant species-crosses, and here too, curiously enough, no male excess was noted for crosses corresponding to those recorded in the present paper, he finds:

		♀	♂
Crosses of *T. picta* and *T. amhersti*	F_1	21	14
	F_2	28	31
	Back-crosses	85	61
Crosses of *G. nycthemerus* and *G. swinhoei*	F_1	2	1
Other Pheasant species-crosses		52	48
	Total	188	155 ♂♂ 45·2 %

On the other hand, this represents the ratio for birds which had assumed adult plumage; and we have seen that owing to the great male mortality, the male ratio of such birds is always considerably less than the total male ratio if all ages are taken into account (see above under Ducks).

[*Postscript.* Since the above was written, Mr Phillips has kindly provided me with further information on his *Thaumalea* crosses, which enables me to classify them according to the Goldschmidtian hypothesis above advanced. The results are as follows:

(*W*, weak = amhersti; *S*, strong = picta; ♀ parent first)

(A)	♀	♂		(B)	♀	♂
$W \times S$	25	26		$S \times W$	23	16
$(W \times S) \times (W \times S)$	8	12		$(S \times W) \times (S \times W)$	19	15
$(W \times S) \times S$	8	7		$(S \times W) \times S$	10	8
$W \times (W \times S)$	7	9		$(W \times S) \times W$	11	4
$W \times (S \times W)$	9	5		$(S \times W) \times W$	5	2
				$S \times (W \times S)$	3	4
				$S \times (S \times W)$	8	7
	57	59			79	56

In (A) ♀ defect is to be expected on my scheme, in (B) not. It will be seen that as a matter of fact (A) gives a close approximation to sex equality, while (B) shows a considerable preponderance of females. *I.e.* the same kind of influence as in the crosses recorded in the present paper seems to be at work, but superposed on a general tendency to a higher ♀ ratio. It should also be recalled that Phillips' birds were only sexed at maturity, which would increase the apparent ♀ ratio.]

Professor Duerden in a letter tells me that it is extremely difficult to obtain accurate sex-ratios in Ostriches, owing to the heavy early mortality and the lateness of external sex-differentiation, and he therefore has no accurate figures for the sex-ratio resulting from crossing the North African and South African Ostriches (Duerden, 1919).

Dr O. E. Plath of the Bussey Institution, Harvard University, writes that the sex-ratio of the canary-finch hybrids raised by Mrs Irelan and himself (Plath, 1922) was close to 1 : 1. This refers to mature or nearly mature birds. The mortality, both before and after hatching, was very high.

Summary.

(1) Species-crosses have been made with 13 species of 7 genera of game birds, mostly pheasants. A number of crosses were wholly sterile (no eggs), others were fecund but infertile.

Of the fertile crosses, very few fertile eggs were obtained in intra-generic crosses. Even in intra-generic crosses the percentage of infertile eggs was always high.

(2) The sex-ratio in the inter-generic crosses could not be determined for lack of numbers. In the intra-generic crosses there is a marked preponderance of males (67·1 per cent. ♂, or more than 200 ♂ : 100 ♀, on 766 specimens). This is in accordance with Haldane's rule.

(3) If we assume that the excess of males is due to sex-reversal during development (as in Goldschmidt's *Lymantria* crosses, but with the complication of circulating sex-hormones added) we should expect that only crosses of one type (those which combine a "strong" X-chromosome with weak or intermediate female-determining factors) should give excess of males, those of the reciprocal type not doing so. When the facts are thus analysed, we obtain a ♂ ratio of 69·5 per cent. where ♀ defect due to sex-reversal is theoretically to be expected, but one of only 54·9 per cent. where ♀ defect is not to be expected. This is strong evidence that the causes of the abnormal sex-ratio are to be sought in

an imbalance of male- and female-determining factors, as in Lepidopteran consecutive intersexuality.

(4) The mortality of the sexes in these crosses is different, that of the males being greater. This excess of male mortality is greatest after hatching but before 6 weeks old. The ♂ ratio is as follows: dead-in-shell, 62·3 per cent.; hatched but dying before 6 weeks, 75·8 per cent.; attaining maturity, 55·1 per cent. This is possibly to be explained by the reversal of sex making the young birds more susceptible to adverse conditions. Cases of malformed gonads were not uncommon in the material.

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FORMATION AND MORPHOLOGY OF
SOLANUM CHIMAERAS.

By C. A. JØRGENSEN and M. B. CRANE.

(With Six Plates and Twenty-three Text-figures.)

CONTENTS.

INTRODUCTION.

THE experiments to which this report relates were begun by C. A. Jørgensen in the spring of 1924 when visiting the John Innes Horticultural Institution. The grafts made by him were cared for by M. B. Crane, who described the chimaeras which subsequently appeared. In April–June 1925 and July–August 1926 Jørgensen again visited the Institution and collaborated in the descriptive work and made cytological observations.

The grafts were made according to the method described by Winkler (1907) and used by him for obtaining chimaeras. The plants were cross-grafted and after union had taken place they were cut across in the grafted region. Some of the adventitious shoots formed from the surface were chimaeras, partly sectorials (Winkler, 1907) and partly periclinals (Winkler, 1908). Adopting the same method it is also possible to induce tetraploidy (Winkler, 1916).

The *Solanum* species used, from which chimaeras were obtained, were: *S. lycopersicum* L. (several commercial varieties: Sutton's Best of All, Balch's Fillbasket, Early Dwarf Red and Large Yellow), *S. sisymbrifolium* Lam., *S. luteum* Mill., *S. nigrum* L., *S. nigrum* var. *gracile* Raddi and *S. guineense* Lam. *S. guineense* is close to *S. nigrum* and often considered to be a variety of it.

<center>Morphology of the Species Used.</center>

In the following account are described the characteristic features of the five species which were used. The descriptions are all based on plants grown under similar conditions in a greenhouse.

(1) *S. lycopersicum* L. The varieties used in the experiments are alike in essential characters. They differ in growth—Early Dwarf Red being smaller and more compact than the others—and in the form and colour of the fruit. The plants easily reach a height of 1–2 m. and have a strong erect stem (Fig. 7). Lateral shoots form, but are of little significance. The type of branching is described later (p. 254). The stem in cross-section is circular or oval. At the base of the leaf insertions it is swollen, light coloured and rich in collenchymatous tissue. The leaves (Fig. 1 *a*) are dark green and are arranged spirally. They have a short petiole and are interruptedly pinnate. The smaller leaflets are simply ovate, the larger ones have a lobed or denticulate outline. With the exception of the terminal leaflet they are asymmetrical, the basal half being the larger. The inflorescences have 7–10 flowers. The sepals are 8–10 mm. long, narrow and green; their number varies from 5 to 7. The petals are triangular-lanceolate, 18–20 mm. long, acuminate, yellow, with a distinct greenish midrib, and are only fused at the base. The number of the petals varies according to that of the sepals. The style is shorter than the stamens. The stamens are syngenesious and form a cone around the style. They are about 10 mm. in length, without filaments, and orange-yellow in colour. Their dehiscence is introrse and made by splits running from the top almost to the base of the anthers.

The fruits of different varieties of *S. lycopersicum* vary considerably in several respects. They differ widely in shape, in the number of loculi, and in colour. Sutton's Best of All and Balch's Fillbasket have rounded fruits. Those of Early Dwarf Red are more oblate and somewhat angular. The colour of the fruits of these three varieties is red. They have red flesh, but the skin is deep yellow. Large Yellow has yellow fruits, both the flesh and the skin of this variety being yellow.

The tomato plant is hairy all over. The hairs are of two kinds: the majority are short, capitate secretory hairs, but in addition there are many long, straight multicellular hairs with a prominent base.

According to Winkler the haploid chromosome number of the tomato is 12, and with this number our own counts agree (Fig. 2).

(2) *S. sisymbrifolium* Lam. This species grows from 0·75 to 1·25 m. high. The plants have a vigorous erect main stem, and on old plants
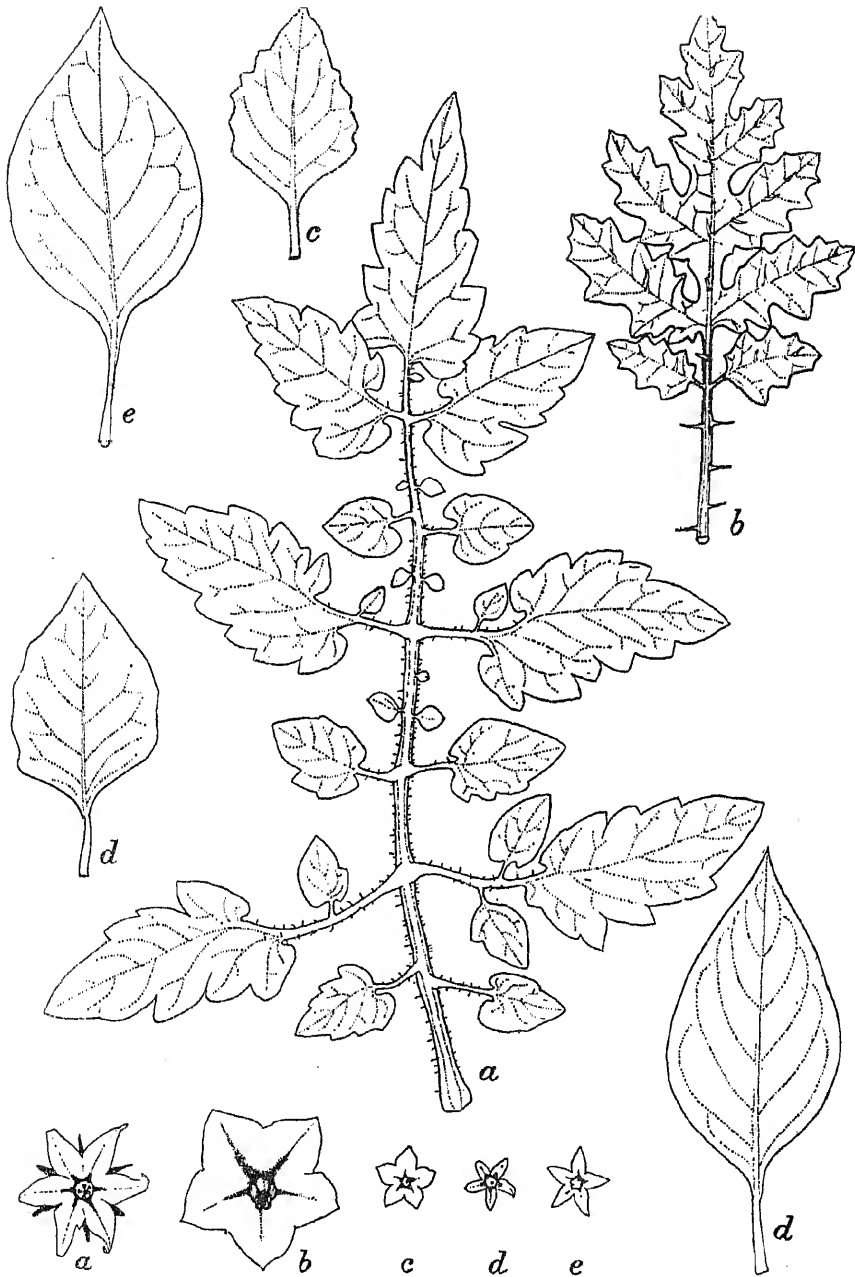
Fig. 1. Leaves and flowers of *S. lycopersicum* (*a*), *S. sisymbrifolium* (*b*), *S. luteum* (*c*), *S. nigrum* and *nigrum* var. *gracile* (*d*), and *S. guineense* (*e*).

side shoots frequently develop and grow taller. The stem is green; angular in cross-section with ribs below the insertion of the leaves.

In growth and ramification it resembles the tomato. The leaves are spirally arranged, and are about 15–20 cm. long and 9–12 cm. wide, with a petiole of 5–6 cm. They are ovate in shape, but owing to deep lobing their outline is irregular, and they are best described as pinnatipartite. The lobes are asymmetrical, the basal part being the larger (Fig. 1 *b*).
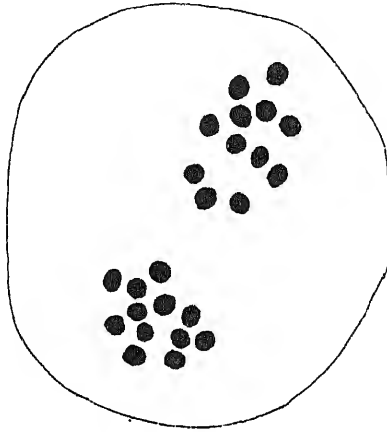

Fig. 2. Tomato, pollen mother-cell in homotype metaphase.

The inflorescences usually have 9–10 flowers. The flowers are conspicuous, measuring 35–40 mm. in diameter. The sepals are small, ligulate, 7–8 mm. long and 2–3 mm. wide. They are green and have thorns at their base. The petals are 16–19 mm. in length and are fused to about half their length (Fig. 1). Their margin is crenate, and when first open they are almost white, turning later to a faint bluish purple colour. The midribs of the petals are yellow and form a central star. The flowers, contrary to those of the other species, are slightly zygomorphic. In connection with this the five stamens are of unequal length, measuring from 7 to 10 mm. The filaments are 1 mm. long. The anthers are quite free and yellow in colour, and dehiscence takes place by a small round opening at the apex. The style is long with a green flattened stigma.

The fruit is mainly enclosed in the persistent sepals; the basal thorny part of the calyx develops considerably during the growth of the fruit. When mature the fruit is 15–20 mm. long, orange-red in colour, ovate and slightly angular.

The plants are very hairy. The majority of the hairs are secretory, but in addition they have many spiny hairs. These spines are from 5 to 10 mm. in length and are confined to the stems, petioles, main veins of the leaves and to the pedicels and sepals of the flowers.

The haploid chromosome number of *S. sisymbrifolium* is 12 (Fig. 3).

(3) *S. luteum* Mill. (*S. tomentosum* Lam.). Under the conditions given the individuals of this species grow to a height of ½ metre, and in habit and type of branching resemble *S. nigrum*. The main stem is short and divides into two branches which again divide irregularly; numerous small shoots are formed. In cross-section the stem is circular or obtuse angular, and the ribs from the base of the leaf insertion are inconspicuous. The stem is green with an occasional faint purple tinge.
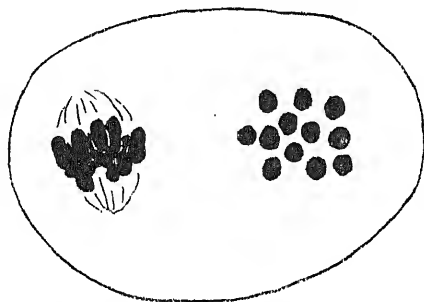

Fig. 3. *S. sisymbrifolium*, homotype metaphase in pollen mother-cell.

The leaves (Fig. 1 c) are spirally arranged but pseudo-opposite on the flowering stems; they are petiolated and ovate in shape and have rather a distinct denticulated outline. There are 5–9 small flowers in an inflorescence. The sepals are small, ligulate, 12 mm. long and green. The petals are broad and triangular, and white in colour with a small yellow eye in the centre of the flower 15–17 mm. in diameter (Fig. 1 c). The stamens have a filament of equal length to the anther (2 + 2 mm.). They are bright yellow and the dehiscence takes place by small openings at the apex. The stamens are quite free and do not form a tube as in the tomato. The fruits are globose, 5–8 mm. in diameter and bright orange in colour. The skin is transparent and in mature fruits the seeds are visible through the skin. The plants are covered with numerous short glandular hairs. The haploid chromosome number of *S. luteum* is 24 (Fig. 4).

(4) *S. nigrum* L. In the greenhouse the individuals of this well-known plant are smaller and less branched than when growing wild. The branching is almost pseudodichotomous, and numerous small shoots

develop from the axils of the leaves (Fig. 7). The main stem is short, circular in cross-section, and occasionally has inconspicuous ribs at the base of the leaves. The stem is bluish purple owing to epidermal anthocyanin. The leaves are arranged as in *S. luteum* and become pseudo-opposite in the flowering stems. Sometimes they are simply ovate, but usually the margin is slightly dentate (Fig. 1 *d*). The inflorescences have 5–11 flowers. The flowers are 8–9 mm. in diameter; the sepals are short and obtuse; the petals are ligulate, almost free, 3–4 mm. in length, and white
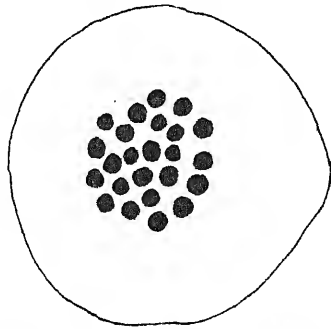
Fig. 4. *S. luteum*, heterotypic anaphase in pollen mother-cell.
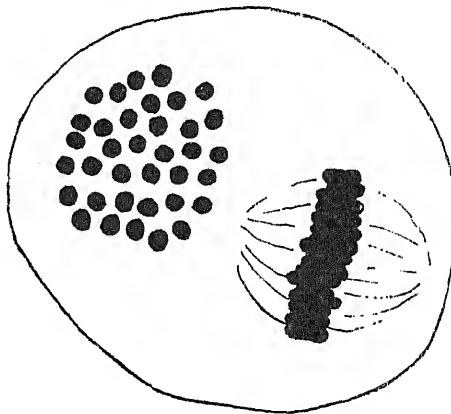

Fig. 5. *S. nigrum*, homotypic metaphase.

in colour with a yellow spot at the base (Fig. 1 *d*). The stamens are 3–4 mm. in length and have short filaments; the anthers are bright yellow and their dehiscence takes place by a circular opening at the tip. There is no staminal tube. The fruits are globose, 5–8 mm. in diameter, shiny and bluish black. The plants are almost smooth, but

a few scattered hairs occur on the stem and on the veins of the leaves. The haploid chromosome number was 36 in Winkler's count, and with this our material agrees (Fig. 5).

In addition to plants of typical *S. nigrum*, plants of *S. nigrum* var. *gracile* Raddi were used in the experiments. They are very close to *S. nigrum*, the most salient differences being a more slender growth and leaves more even in outline (Fig. 1 *d*). The haploid chromosome number is 36, as in *S. nigrum*.

(5) *S. guineense* Lam. This species is often looked upon as a variety of *S. nigrum* (cf. *Index Kewensis*). It is however a distinct type, and is for convenience considered by us as a species. The plant is in all parts larger than *S. nigrum* and often reaches a height of 0·75 m. The main
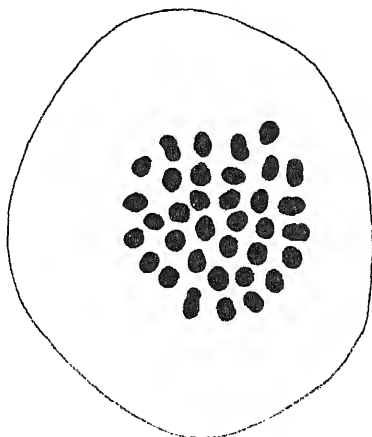
Fig. 6. *S. guineense*, heterotype metaphase in pollen mother-cell.

stems are strong and angular, with prominent ribs and furnished with thorny hairs. The larger stems are bluish in colour, the smaller ones green. The type of branching is as in *S. nigrum*. The leaves are deep green, large, ovate and acuminate and have a simple outline (Fig. 1 *e*). The inflorescences and flowers are like those of *S. nigrum*, only larger. The diameter of the opened flowers is 12–15 mm. The anthers when young are a yellowish brown, and turn a deeper brown with age, not yellow like those of *S. nigrum*. The style is the same length as the stamens. The fruits are globose, 8–12 mm. in diameter, and intensely black when ripe. The plants have very few hairs, those that are present occurring mostly on the stems and veins of the leaves. The hairs are short, and those on the stems have the character of small thorns.

The haploid chromosome number of *S. guineense* is 36 (Fig. 6), the same as that of *S. nigrum*.

The branching of the flowering shoots of Solanums is sympodial. In the varieties of tomato used in these experiments and in *S. sisymbrifolium* this is only seen in the peculiar irregular way the inflorescences are adnated to the apparent main stem and their relative positions displaced; yet the leaves have a uniform spiral arrangement, and lateral growth appears in a regular order. The other species are more complex

Fig. 7. Shoots of *S. nigrum* (left) and tomato (right).

and show a further development of this character, in that each alternate leaf on the flowering shoots is carried up the main axis to the level of the leaf above, and appears as a smaller leaf opposite to the upper larger one.

In the table on p. 255 the most salient characters of the species are summarised.

	S. lycopersicum	S. sisymbrifolium	S. luteum	S. nigrum	S. guineense
Approximate height	2 m.	1 m.	0·5 m.	0·5 m.	0·75 m.
Leaves	Spirally arranged, lobed, interruptedly pinnate	Spirally arranged, deeply lobed, pinnatipartite	Spirally arranged, but pseudo-opposite on the flowering stems, petiolated, ovate with a denticulated outline	Spirally arranged, pseudo-opposite on the flowering stems. Ovate, margin usually dentate	Spirally arranged, ovate and acuminate, with a simple outline
Inflorescence	7-10 flowers	9-10 flowers	5-9 flowers	5-11 flowers	5-11 flowers
Petals	Triangular-lanceolate 18-20 mm. long, acuminate, yellow with a greenish midrib. Fused at the base	16-19 mm. long. White turning to a bluish purple. Midrib yellow. Fused to about half their length, margin crenate	Triangular 7-9 mm. long. White with a small yellow spot at the base. Fused to about half their length. Bright yellow	Ligulate, 3-4 mm. long. White with a small yellow spot at the base. Almost free	Similar to those of S. nigrum but larger
Sepals	8-10 mm. long, narrow and green	7-8 mm. long, ligulate, green. Spines at base	12 mm. long, green and ligulate	Short and obtuse	Short and obtuse
Style	Shorter than stamens	Long, with a green flattened stigma	Same length as stamens	Same length as stamens	Same length as stamens
Stamens	Syngenesious, 10 mm. long, without filaments. Orange-yellow	Free, unequal in length. Yellow	Free, stamens and filaments of equal length 2+2 mm. Bright yellow	Free, short filaments. Bright yellow	Free, short filaments. Dark brown
Hairs	Hairy all over. Hairs of two kinds, short and secretory; long and multicellular	Covered with secretory hairs and numerous spines. Many of the hairs are stellate	Covered with numerous fine glandular hairs and a few long ones	Few scattered hairs. They mostly occur on the stems and veins of the leaves	Few scattered hairs. On the stems they resemble small thorns
Fruit	Many varietal differences. Red or yellow flesh. Yellow or transparent skin	Enclosed in the persistent spiny calyx. Ovate, 15-20 mm. long. Orange-red	Globose, 5-8 mm. in diameter, bright orange. Skin transparent	Globose, 5-8 mm. in diameter, shiny and bluish black	Globose, 8-12 mm. in diameter, intensely black
Chromosomes	$n=12$	$n=12$	$n=24$	$n=36$	$n=36$

S. nigrum var. gracile has slenderer growth than S. nigrum and leaves more even in outline. In all other respects it is very similar to S. nigrum.

FORMATION AND DEVELOPMENT OF CHIMAERAS.

As previously mentioned, the method employed by us for producing the chimaeras was the same as that used by Winkler (1907). The main shoots of young and vigorous plants of the two species concerned were exchanged by grafting, and after they had united the plants were cut across at the point of union and all subsequent axillary shoots removed. The plants were "cleft-grafted," and when cut down the tissues were exposed on the cut surface as shown in Fig. 8—the white area representing the stock, and the black area part of the scion.
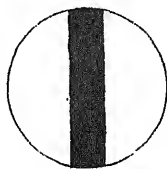
Fig. 8. Cross-section of grafted stock.
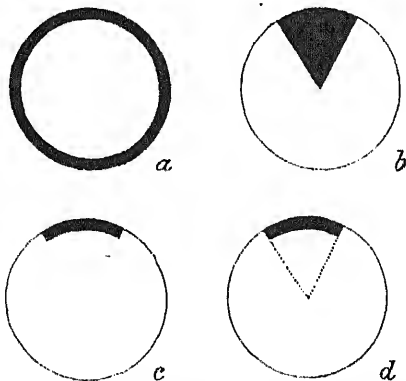
Fig. 9. Cross-sections of stems of different types of chimaeras:
a, periclinal; *b*, sectorial; *c* and *d*, mericlinal.

Callus is produced by the cut surface and from this young shoots differentiate. Most of these are pure, and consist of uniform tissue of one or other of the species used, but from parts of the surface where the two tissues unite shoots occasionally arise in the formation of which both specific tissues take part. The chimaeras formed are either periclinal or sectorial. The last term is however used for different types of chimaeras, and before we describe the chimaeras obtained from the experiments a brief digression on this point is necessary.

A periclinal plant has its interior completely covered by a skin of another type which may be one or more cell layers thick. Fig. 9 *a* shows a cross-section of a young stem of such a plant. The leaves are all alike and to some extent intermediate in shape between those of the two species of which the plant is composed. When these plants are fertile their progeny reproduce only the character of that forming the sub-epidermis.

In a sectorial plant a sector (of the stem) is formed of tissue of another species or type (Fig. 9 *b*). Such a plant will have three types of leaves, some of the type of one species, some of the other and a few sectorial. The sectorial leaves form in the region of the stem where the two tissues meet, the two parts being divided by a longitudinal line.

The third type is shown in Fig. 9 *c* and *d*. These plants have the appearance of sectorials and were generally considered as sectorials also by Winkler, although his definition (1914) of sectorials as plants "bei denen die verschiedenartigen Zellen im Vegetationspunkt durch Längsflächen voneinander getrennt sind" is quite in accordance with ours. A plant however that has on the stem a stripe of tissue, a few cell layers thick, of another species, is not a sectorial in the true sense of the word, although it has hitherto been looked upon as such owing to its external appearance. Fig. 9 *d* shows how this misconception may have arisen. The type is actually closer to the periclinals and is best looked upon as an incomplete periclinal. As these plants, however, represent a distinct type, C. A. Jørgensen has proposed for them the name "mericlinal." Fig. 9 *c* is a cross-section of a young stem of a mericlinal plant. The leaves will be partly uniform tissue of one species, partly periclinal, and partly (from the region where the tissues fuse) mericlinal.

Some well-known examples of periclinal plants are *Cytisus Adami*, the *Crataego-Mespili*, many variegated plants, *Bouvardia* and *Pelargonium*; the structure of the two last-named plants was made evident by root-cutting experiments (Bateson, 1916). Sectorials of the type to which the term is restricted in this paper are of very rare occurrence. The plant first obtained by Winkler (1907) appears to have been one, and a few Pelargoniums are also known. The large majority of plants, however, hitherto mentioned in literature as "sectorials" are mericlinals. Chimaeras of this type are quite common, sometimes as whole plants, often only as shoots or even as flowers or fruits.

The number of cross-graftings made was 250. Adventitious shoots were formed abundantly throughout the summer and autumn. Even in the following spring, almost a year after the grafts were made, some

of the stocks continued to produce adventitious shoots. It is, however, noteworthy that the original callus produced on the cut surface of the grafted plants commonly developed irregularly. The callus on the separated parts of the stock—the white areas shown in Fig. 8—and that on the scion frequently failed to coalesce. This was probably due to shrinkage occurring after the plants were cut down, as a slight cleavage frequently developed between the different tissues. After the first batch of adventitious shoots was removed the surface of the original callus on the majority of the plants was shaved off with a razor. More callus tissue was formed and a greater unity between the surface of the specific tissues resulted. Young shoots again differentiated, and it was among this second batch that the majority of the chimerical forms were obtained. Quite a number of periclinals appeared, but we did not succeed in obtaining a complete series of periclinals between any of the species, such as Winkler obtained and described from his *S. lycopersicum-nigrum* experiments.

In addition to the five species previously mentioned, *S. lycopersicum, S. sisymbrifolium, S. luteum, S. nigrum* and *S. guineense*, a number of other species were used in the experiments, but although they were subjected to the same treatment they failed to produce shoots from which chimaeras could be established. In a few cases shoots formed which had small mericlinal areas, but they soon developed into growth which was pure for one of the species used.

The chimaeras obtained by us are shown in the following scheme in which the first name indicates the interior and the second the skin. The number of layers in the skin is indicated by the Roman numeral. Winkler gave his plants new names, but this was before the real nature of the plants was known, and with our present knowledge we think it best to designate the plants as in this paper.

S. lycopersicum (tomato)	—	*S. lycopersicum-nigrum* (ii)	—	*S. nigrum*
,,	*S. lycopersicum-guineense* (i)	*S. lycopersicum-guineense* (ii)	—	*S. guineense*
,,	*S. lycopersicum-luteum* (i)	*S. lycopersicum-luteum* (ii)	*S. luteum-lycopersicum* (i)	*S. luteum*
S. sisymbrifolium	—	—	*S. nigrum-sisymbrifolium* (i)	*S. nigrum* var. *gracile*

The majority of the adventitious shoots formed were of course pure, *i.e.* wholly composed of one or other of the two species used to form the composite cross-grafted plants. The others were from the start mericlinal, and *it is noteworthy that in these experiments a complete periclinal*

shoot was never observed to arise directly from the cross-grafted plants. Winkler does not discuss in detail the formation of his chimaeras, but we think this point is of some importance. With Fig. 8 in view it is evident that when a callus develops from the surface, the shoots formed at the juncture of the two tissues will have a structure of a sectorial type if both tissues take a substantial part in their formation, but as a rule the part played by one of the component tissues is a minor one, and consequently the composite structure that results is usually the meri- clinal. In Plate XI, figs. *a–d*, the base of the stem of four primary shoots is shown. In all four the mericlinal structure is evident. The plant in Fig. *a* grew into pure tomato, but on the stem a stripe of *luteum* tissue is seen. That this *luteum* stripe is only one cell layer in thickness is clearly indicated by the side shoot which developed, and became a periclinal of the type *S. lycopersicum-luteum* (i). In Fig. *b*, the stripe is *guineense* tissue over tomato, and again a one-layered periclinal side shoot formed. The stem of the plant in Fig. *c* has a stripe of tomato tissue on which the long conspicuous tomato hairs are evident. All the interior of this plant is tomato, and the plant soon turned into a periclinal of the type *S. lyco- persicum-guineense* (i). Fig. *d* is also a good example of a mericlinal, and the fact that the *guineense* stripe on the stem is only composed of one layer is evident from the periclinal axillary shoots. In Solanums the mericlinal condition is not stable; this can only occur when the cell divisions in the meristematic tissue of the stem are regular. In Solanums, as mentioned before, the branching is sympodial, the successive main axes terminating in inflorescences and being replaced by new ones arising laterally. When an inflorescence develops, a new growing-point from which the next stem arises is formed from that part of the stem which is opposite the inflorescence. Consequently, if this part happens to be covered by the skin of the other species a periclinal is formed, otherwise the main shoot will be pure for one of the species used. The sympodial type of branching is thus in our opinion of fundamental significance in the formation of periclinals from the callus. In a species with monopodial growth, periclinals are not likely to be produced in this way, but only as axillary shoots from the covered part of the mericlinal main shoot.

Morphology of the Chimaeras.

The history and structure of the chimaeras obtained in these experi- ments are given in the following pages. They are referred to in the same order as in the scheme detailed on p. 258, the one-layered forms being mentioned first, then the two-layered, etc.

1. *S. lycopersicum-guineense* (i). From cross-grafting tomato Early Dwarf Red on *S. guineense* and *vice versa*, two plants of this type were obtained. Several individuals were formed later, *e.g.* the shoots shown on Plate XI, figs. *b* and *d*. As mentioned in the introduction, *S. guineense* is very similar to *S. nigrum*, consequently these chimaeras do not differ much from Winkler's *S. Koelreuterianum*. Both plants were mericlinal at the beginning, and one is figured on Plate XI, fig. *c.* Part of the first leaf was wholly tomato, and part had one layer of *guineense* over tomato; the third and all subsequent leaves were periclinal. The plants are in most characters similar to tomato. The most salient difference is the
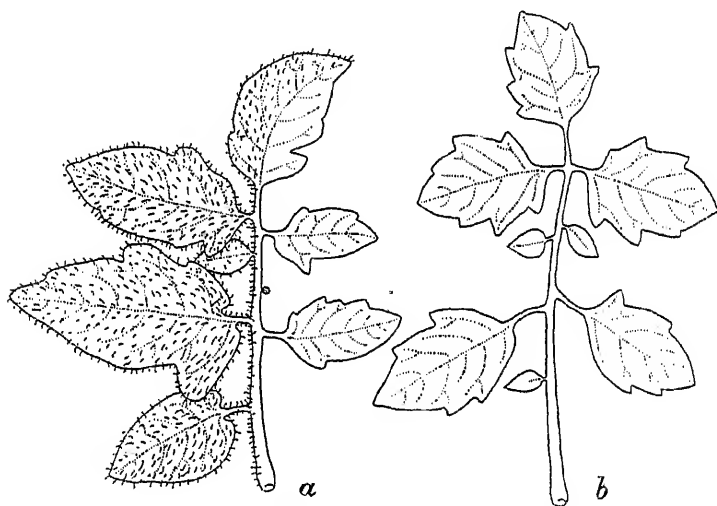
Fig. 10. Leaves of *S. lycopersicum-guineense.*

lack of hairs, the chimaeras having the almost smooth skin of *S. guineense* (Plate XII, fig. 1). The plants have in many respects the appearance of a seedling dahlia; their growth is noticeably slower than that of the tomato, but the thick erect stem and the type of branching is like that of tomato. The leaf differences are best seen on a mericlinal leaf such as that shown in Fig. 10 *a* where the left side is pure tomato and the right is covered with a one-layered *guineense* skin. On this side the leaflets àre smaller, more sharply dentate and fewer in number. The asymmetrical terminal leaflet is characteristic. A typical leaf is shown in Fig. 10 *b*. The colour is dark green; the inflorescences are mostly opposite a leaf and have the same number of flowers as the tomato. The number of sepals is usually six or seven, their length is 5–6 mm. and

they are broader than those of the tomato. The petals are 8–10 mm. long, and the opened flowers have a diameter of 18 mm. In shape they are like tomato petals, but they differ in colour. They have broad white margins, but the middle of the lamina from base to tip is yellow (Plate XVI, fig. 2, *b* 2). The stamens are widely separated and have a short filament, but the anthers are similar to those of the tomato in shape. The pistil is large, rather short, and persists on the young growing fruit. The fruits are like tomatoes in shape except for the apical point formed by the persistent style (Fig. 11). They are, however, quite different in colour, being blackish purple mottled with dull red. The seeds are very tiny and non-viable. The mucilaginous layer is black and the central part is red. The chromosome number in the reduction division of these plants was not counted in 1925, and in the winter of 1925–6 the plants of this type died.
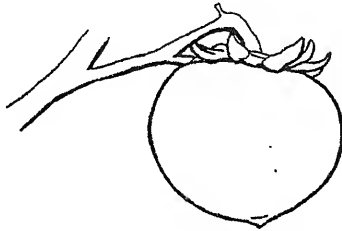
Fig. 11. Fruit of *S. lycopersicum-guineense* (i).

2. *S. lycopersicum-luteum* (i). Plants of this type arose several times in the experiments. The plant described here is shown on Plate XII, fig. 2, and resulted from grafted *S. luteum* on tomato Sutton's Best of All. The young shoots from which the plants of this type developed were all mericlinal at the base, but they soon developed into the periclinal condition. In general the plants resemble the tomato, having a definite main stem, the same type of branching, erect growth and divided leaves. They are also hairy, but the coat consists of the short delicate hairs of *S. luteum*. The leaves (Fig. 12), although divided, differ considerably from tomato leaves. They often have only one pair of side leaflets and never more than two, whereas the tomato has three or more. The leaflets are also broader, more sharply dentate and thicker than those of tomato.

In their early stages the plants of this type are characterised by their extremely slow growth, but they continue to grow, and under favourable conditions have eventually reached a height of 2 m. The plants have not yet flowered; minute inflorescences occasionally form,

but they always fall long before the flowers develop. As a result of the internal diversity between the two components of the plant, areas of cork tissue form on the stem where these diminutive inflorescences appear.

From analogy with the previous type it is evident that the *luteum* skin of this type is only one layer thick. No cytological analysis has been made at present.
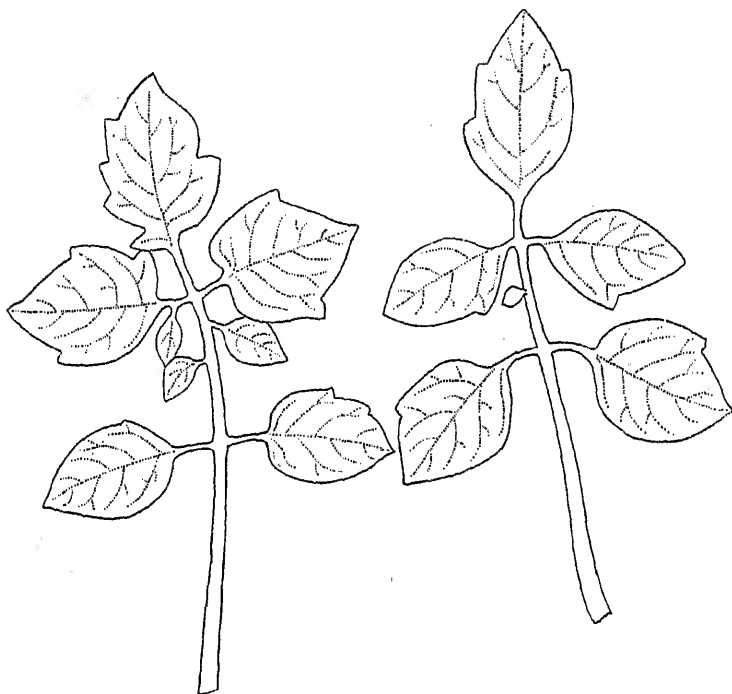
Fig. 12. Typical leaves of *S. lycopersicum-luteum* (i).

3. *S. nigrum-sisymbrifolium* (i). The *nigrum* plants in this combination are of the var. *gracile* Raddi (see Fig. 1). The chimaera arose several times in the experiments, and is a very interesting and quite a decorative plant. In growth it is intermediate between the two species of which it is composed, being more branched than *sisymbrifolium* and taller than *nigrum* (Plate XIII, fig. 1). The stems are dark green with an occasional bluish anthocyanin tinge. The ramification is like that of *nigrum* and the leaves are pseudo-opposite. Many short lateral shoots develop, which, after producing a few leaves, terminate in an inflorescence. The inflorescences on the main shoot frequently arise at the node between the leaves.

The leaves (Fig. 13) are petiolated, ovate, similar to those of *gracile* in shape, but are characterised by their sharply dentate or lobed margins. The inflorescences usually have 2–6 flowers. The flowers are peculiar and far from being intermediate between the species. The sepals are short
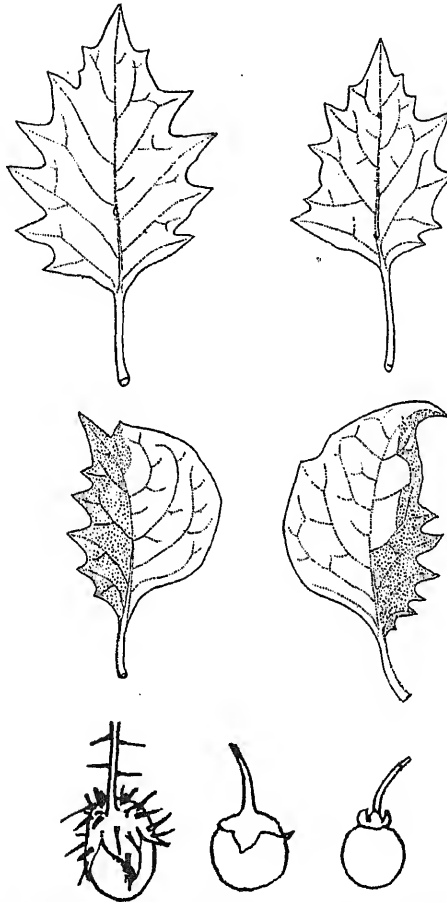
Fig. 13. Leaves of *S. nigrum-sisymbrifolium* (i), fruits of *S. sisymbrifolium* (left), *S. nigrum-sisymbrifolium* (i) (centre), and *S. nigrum* var. *gracile* (right).

(3–4 mm. long by 1–1·5 mm. wide), ligulate and green. The petals are almost spathulate with a short stalk and a large oval lamina 10–12 mm. long by 6–7 mm. wide. They are free almost to the base like those of *S. nigrum*, and the lateral parts being mainly composed of *S. sisymbrifolium* tissue grow to a considerable size. When first open they are

white, but they soon develop a bluish purple colour, a characteristic of
S. sisymbrifolium. The filaments and anthers are approximately equal
in length; the anthers are free, 4–5 mm. long, bright yellow, dehiscing
through an apical pore. The pistil is very long, curved and a little
flattened on the surface.

The plants are very hairy. The hairs are secretory, and spines occur
which are distributed as in *S. sisymbrifolium*. The spines are, however,
greatly reduced and though at the base they are almost as large as those
of *S. sisymbrifolium* they are very short. This chimaera is less stable
than any of the forms we have obtained. Frequently the core breaks
through the skin and pure *S. nigrum* var. *gracile* shoots ultimately
appear. This breaking commonly occurs also in the inflorescence, conse-
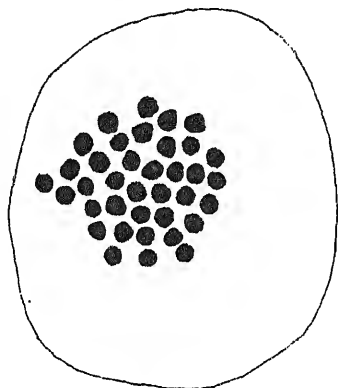
Fig. 14. Heterotypic metaphase in pollen mother-cell of
S. nigrum-sisymbrifolium (i).

quently many fruits form which in varying degree are mericlinal. The
fruits are black, and are shown in Fig. 13. The sepals tend to enclose
the fruit as in *sisymbrifolium* (*nigrum* sepals curve backward away from
the fruit). The fruits contain a number of well-developed seeds which
on germination give, as would be expected, plants which are *S. nigrum*
var. *gracile*.

The reduction division of the pollen mother-cells shows 36 chromo-
somes as in *S. nigrum*. This and the breeding results show that the
S. sisymbrifolium skin is only one layer thick.

4. *S. lycopersicum-nigrum* (ii). This chimaera resulted from graft-
ing tomato var. Balch's Fillbasket on *S. nigrum*, and corresponds to
Winkler's *S. Gaertnerianum*. Plate XIV, fig. 1, shows the plant at an early
stage, and it is evident that both in shape and in the absence of hairs

the leaves resemble *S. nigrum* much more than the tomato. They are however curiously buckled, which is doubtless the result of differential growth of the two component tissues. In the two-layered periclinals in which the species forming the external component has a comparatively small and simple leaf, and the internal one a large compound leaf, the phenomenon of buckling seems to be a prominent characteristic, *e.g.* it is very much marked in *S. lycopersicum-luteum* (ii) (see p. 267). *S. lycopersicum-nigrum* (ii) shows erect growth with a vigorous main stem of which the surface is uneven and dark green. The type of branching
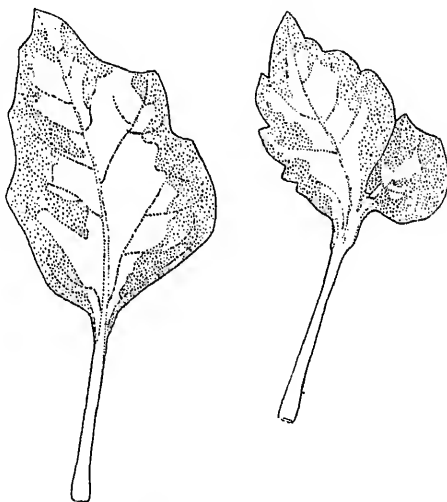
Fig. 15. Leaves of *S. lycopersicum-nigrum* (ii).

resembles that of tomato, and the inflorescences appear at the nodes. The leaves, although nearer to *S. nigrum* than *S. lycopersicum*, are almost monstrous and very irregular in shape. The young leaves are uniformly green, but as they develop the lamina bleaches and yellowish areas are formed. This change is due to diminution in the size of the chloroplasts. Plate XIV, fig. 2, shows that the distribution of these yellowish areas depends upon the main venation of the leaf—see also *S. lycopersicum-guineense* (ii). Winkler does not describe this peculiarity, it therefore seems probable that it did not occur in his plants. This suggests that chimaeras of the same structural combination are not always identical. Such variations are probably attributable to differences between the different varieties of *S. lycopersicum* used.

The inflorescence is similar to that of tomato and has 5–8 flowers. The shape of the flower is perhaps nearer to *S. nigrum*, but in general it is intermediate between the two species (Plate XVI, fig. 2, *c* 1). The petals are white, but in the centre they have a faint yellow stripe. The supernumerary leaflets are derived from the tomato. Although the plant was healthy and grew vigorously in the summer of 1925 the flowers dropped without developing fruits. The flowers on Winkler's plants of this type failed to form fruits at first, but subsequently they produced some with viable seeds which gave pure *S. nigrum* seedlings.

The haploid chromosome number in the reduction division of the pollen mother-cells is 36 (Fig. 16), the same as in *S. nigrum*, showing that in this chimaera the two outermost layers at least are composed of *S. nigrum* tissue.
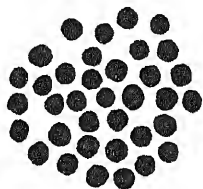
Fig. 16. Heterotypic metaphase of *S. lycopersicum-nigrum* (ii).

5. *S. lycopersicum-luteum* (ii). Several plants of this type were formed from the callus produced on the cut surface of the cross-grafted plants. They all began as mericlinals, as shown in the plants figured in Plate XIV, fig. 3. The two lower leaves are partly composed of one layer of *luteum* over tomato and partly of pure *luteum*, while other leaves seen behind are wholly composed of *luteum* tissue. The leaf in front has, however, two layers of *luteum* over tomato, and the plant ultimately formed a chimaera of the type *S. lycopersicum-luteum* (ii). Plate XV, fig. 1, is a photograph of the same plant at a much later stage, and shows that the main shoots have remained constant to the *lycopersicum-luteum* (ii) type, but from the base of the main stem shoots of another type, *S. luteum-lycopersicum* (i), have developed. On the plant shown in Plate XV, fig. 2, a tomato leaf is seen at the base, and a shoot of pure *S. luteum* has broken out from the main stem. In both of these plants it is evident that the development of shoots of a different type is due to their primary mericlinal structure.

The plants of this type are in size and growth somewhat intermediate between their two components, but although produced at approximately the same time and subjected to similar treatment they differ in habit.

Two of them, in which the tomato is the var. Balch's Fillbasket, are identical, but in the others the variety of tomato which composes the core is Sutton's Best of All, and these differ in having a more vigorous and erect growth and fewer axillary shoots. They also fail to produce flowers (Plate XV, fig. 2). This shows that the variety of the tomato may have a considerable influence on the appearance and structure of the resulting chimaeras, and doubtless this accounts for some of the differences between the chimaeras described in this paper and those of Winkler.
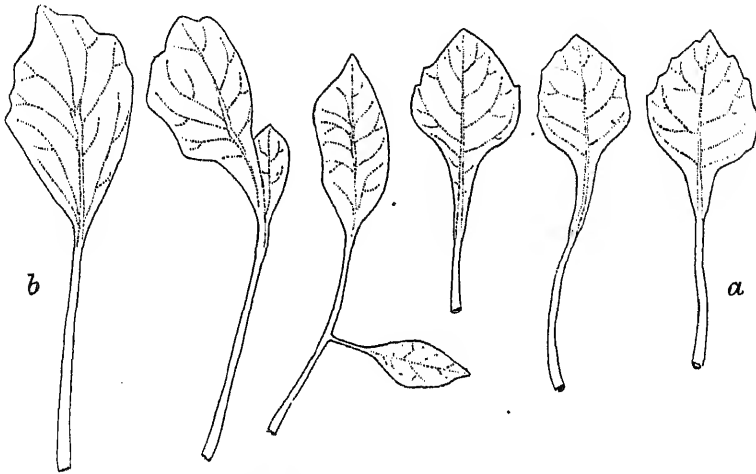
Fig. 17. Leaves of *S. lycopersicum-luteum* (ii).

The skin of the plants is covered with the comparatively short hairs of *S. luteum*, and in general the plants are nearer to this species in appearance. The main stems of the plant are rather thick and cylindrical, and branch comparatively freely, but the branches are more stiff and erect. In many characters the leaves are like those of the corresponding chimaeras between *S. lycopersicum* and *S. nigrum*. They are normal green, have long petioles and buckled laminas. The venation is peculiar (Fig. 17 *a* and *b*), the main bundle only reaches the middle of the lamina and then divides into several branches approximately equal in size. The leaves of the plants in which the tomato component is Balch's Fillbasket are always of the shape illustrated in Fig. 17 *a*, but in those containing Sutton's Best of All subdivided leaves, or leaves with a single free leaflet, are not uncommon (Fig. 17 *b*).

The shoots with the typical buckled leaves are not able to form

flowers, and such plants as that shown in Plate XV, fig. 2, never attempt to flower. On other plants, however, shoots are formed with flat leaves which have very long petioles, and these produce flowers and fruits. Their inflorescences, flowers and fruits are identical with those of *S. luteum*.

Fig. 18. Heterotypic metaphase of *S. lycopersicum-luteum* (ii).
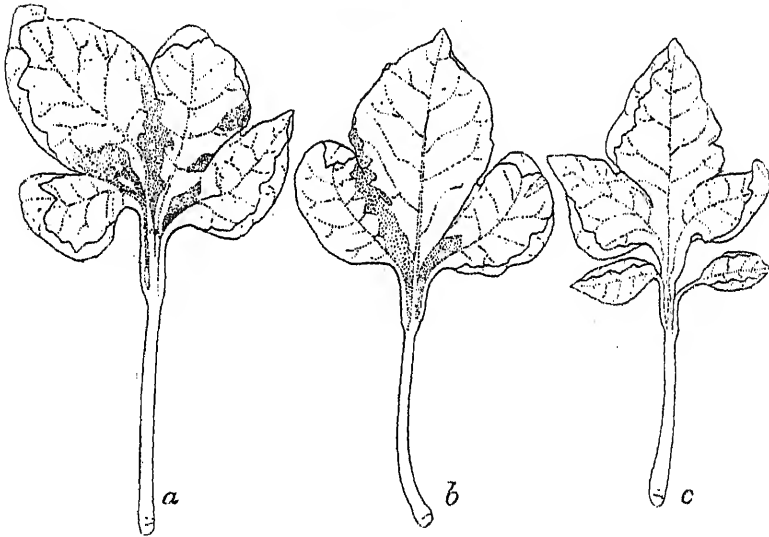
Fig. 19. Leaves of *S. lycopersicum-guineense* (ii).

From analogy between this type and the *S. lycopersicum-nigrum* (ii) chimaeras, it is clear that in these plants two layers of *S. luteum* are outside a tomato core. The chromosome number in the reduction division of the pollen mother-cells is 24 (Fig. 18), as in *S. luteum*.

6. *S. lycopersicum-guineense* (ii). This plant was obtained from grafting *S. guineense* on tomato Early Dwarf Red and as shown in Plate XV, fig. 4, it began as a mericlinal. The first leaf is divided like those of tomato, and is composed of one layer of *S. guineense* over tomato; all the following leaves have two external layers of *S. guineense*. Except

for the practically smooth surface of *S. guineense* the chimaera is nearer to tomato. The stem is thick, erect and somewhat uneven on the surface, and the leaves are spirally arranged as in the tomato. The shape of the leaves is peculiar, the lower ones being three lobed with a large median part (Fig. 19 *a* and *b*) while the others usually have a pair of quite free leaflets (Fig. 19 *b* and *c*). On the leaves stripes of a peculiar shiny green are often evident, and are represented in the figures as dotted areas. The leaves are buckled, but the upper surface is concave instead of convex as in the two-layered types previously described.

The inflorescences and flowers are like those of *S. lycopersicum-nigrum* (ii) and so far no fruits have formed.
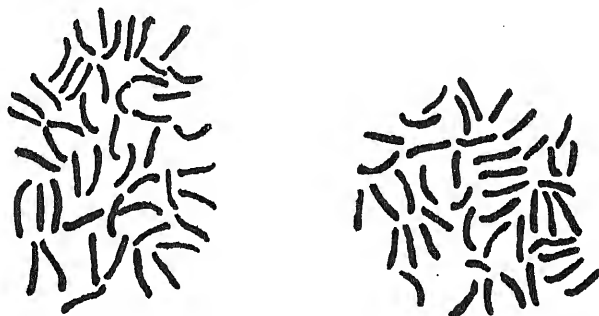
Fig. 20. Metaphase-plates from roots of *S. lycopersicum-guineense* (ii). About 48 chromosomes.

Cytological examination of the root tips show that the tomato core of this chimaera has about 48 chromosomes—see Fig. 20—instead of the normal diploid 24 of the tomato. Since all the tomato plants used in the experiments were normal diploids we must conclude that doubling of the chromosome number took place in the meristematic callus tissue formed on the cut surface of the cross-grafted plants.

7. *S. luteum-lycopersicum* (i). The two axillary shoots from the meri-clinal base of the plant shown in Plate XV, fig. 1, gave rise to chimaeras of this type, and another rose independently from the cut surface of a cross-grafted plant.

The plants are in growth very similar to *S. luteum*, but readily distinguished from it by their long tomato hairs (Plate XVI, fig. 1). The stems are slender and branch freely, and in colour are bright green, although Balch's Fillbasket, the tomato which forms the skin, develops much anthocyanin. The leaves are ovate and of about the same size as those in *S. luteum*. The sepals are green, small and linear. The petals are

lanceolate-linear and free almost to the base, whereas those of *S. luteum* are united up to half their length. They are uniformly yellow, but not quite so deep a yellow as those of the tomato. The filaments and anthers
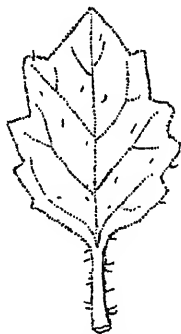
Fig. 21. Leaf of *S. luteum-lycopersicum* (i).
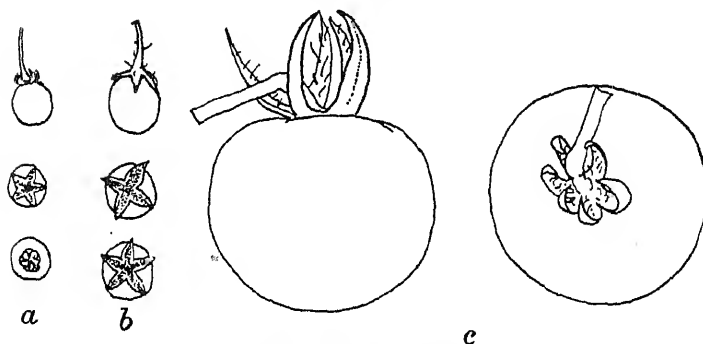
Fig. 22. Fruits of (*a*) *S. luteum*, (*b*) *S. luteum-lycopersicum* (i), and (*c*) *S. lycopersicum*.

Fig. 23. Metaphase in pollen mother cell of *S. luteum-lycopersicum* (i).

are about equal in length; they remain together but are not fused like those of the tomato. This is the only type we have obtained with the external layer composed of tomato, and the only chimaera which has the strong characteristic smell of the tomato. This chimaera produces

fruit freely. The fruits are a little larger than those of *S. luteum* and are opaque yellow in colour, the opacity being due to the tomato skin. The sepals adhere to the fruit, whereas in the tomato and in *S. luteum* they bend sharply backwards away from the fruit (Fig. 22). The fruits contain very poorly developed seeds of which the majority fail to germinate, but about 200 seedlings have been raised. In the early stages they are slender and very slow-growing, but later they develop into normal *S. luteum* plants. The reduction division of the pollen mother-cells shows 24 chromosomes (Fig. 23) as in *S. luteum*, indicating that in this chimaera there is only one epidermal layer of tomato.

SOMATIC INSTABILITY OF PERICLINAL CHIMAERAS.

After formation of the various chimaeras periclinal shoots were taken from them for propagation, and a number of plants of each type were raised and grown on. Many of the plants have remained constant to their periclinal structure, but some have developed growth of a different type and the following summarises the somatic rearrangements that have occurred during the two years they have been under observation.

Periclinal chimaera	Somatic rearrangement
S. nigrum var. *gracile-sisymbrifolium* (i)	Commonly gives pure *S. gracile*
S. lycopersicum-guineense (i)	Has on one occasion given pure *S. lycopersicum*
S. lycopersicum-luteum (i)	Has on three occasions given pure *S. lycopersicum*
„ (ii)	Has twice given *S. lycopersicum-luteum* (i) and once pure *S. lycopersicum*
S. luteum-lycopersicum (i)	Frequently gives pure *S. luteum*
S. lycopersicum-luteum (ii)	Frequently gives pure *S. luteum*

It is noteworthy that six of these somatic changes are due to the loss of one or more layers of the species forming the skin. The seventh example is however peculiar and more complex, involving the development of *S. lycopersicum-luteum* (ii) into pure *S. luteum*. It must be noted however that this periclinal first develops transitional shoots with flat, petiolated and comparatively small leaves (see Plate XV, fig. 3); and although no definite statement can be made the general appearance of such shoots suggests that they may possess three or more external layers of *S. luteum*.

Some of the periclinals of the type *S. nigrum-sisymbrifolium* (i) are more stable than others, cf. figs. 1 and 2 in Plate XIII. The differences in the behaviour of these chimaeras are probably due to differences that occur within *S. sisymbrifolium*. The plants of *S. luteum*, *S. guineense*,

S. nigrum and *S. nigrum* var. *gracile* used in the experiments were extremely uniform, but those of *S. sisymbrifolium* varied in minor respects, some being much more spiny than others, while differences also occurred in the lobing and size of the leaves as well as in the depth of the flower colour.

A paper on the anatomy of the chimaeras, together with further details of morphological interest will be published later by C. A. Jørgensen.

Both the authors acknowledge the deep debt of gratitude they owe to the late Mr W. Bateson for the encouragement he constantly gave them. C. A. Jørgensen is also indebted to the Council of the John Innes Horticultural Institution and to the Councils of the Carlsberg and Rask-Örsted Foundations in Copenhagen for grants enabling him to visit the Institution during three consecutive summers in order to continue the investigations.

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EXPLANATION OF PLATES.

PLATE XI.

Figs. *a–d.* Showing the mericlinal condition of the basal part of four adventitious shoots.
Fig. *a.* Combination of *S. lycopersicum* and *S. luteum.*
Figs. *b–d.* Combination of *S. lycopersicum* and *S. guineense.*

PLATE XII.

Fig. 1. Young plant of *S. lycopersicum-guineense* (i).
Fig. 2. Young plant of *S. lycopersicum-luteum* (i).
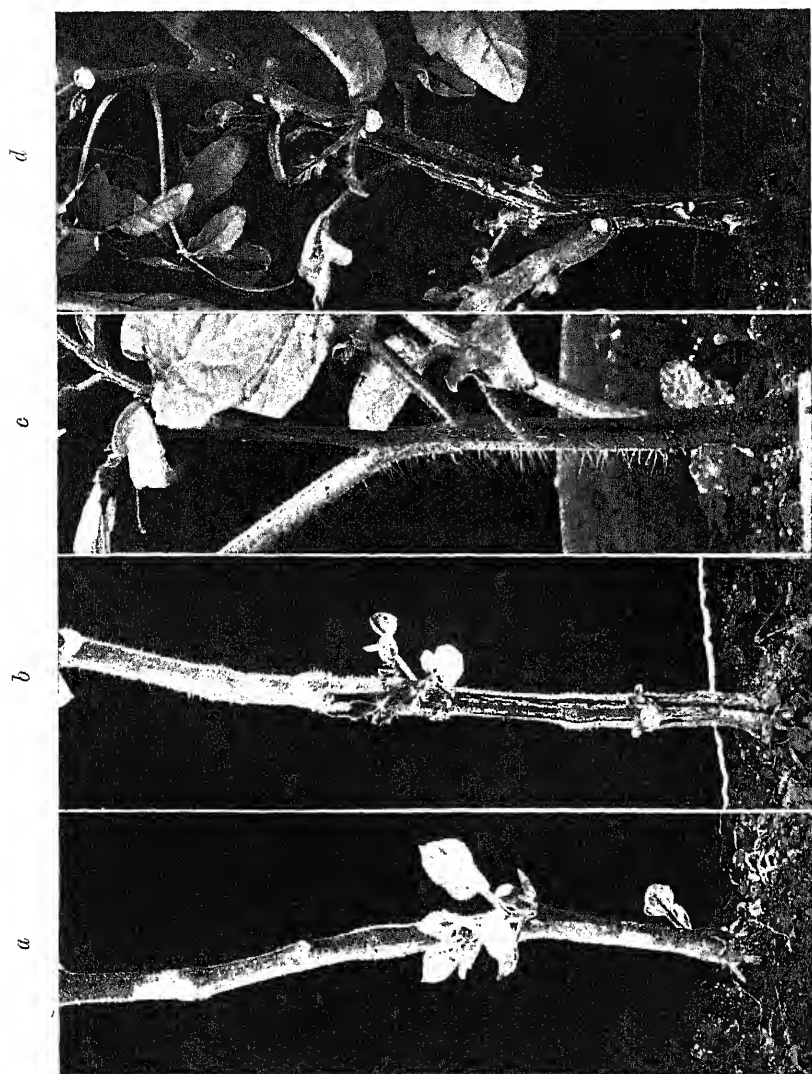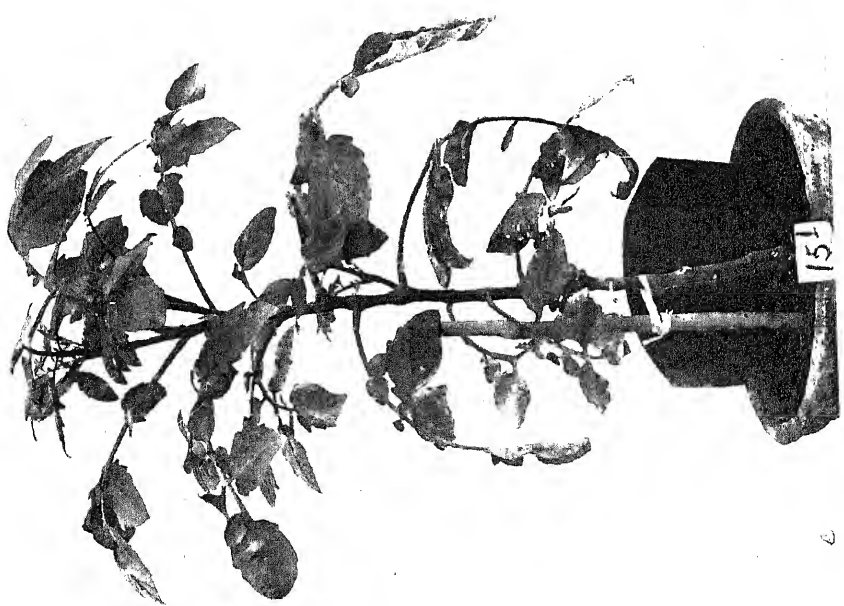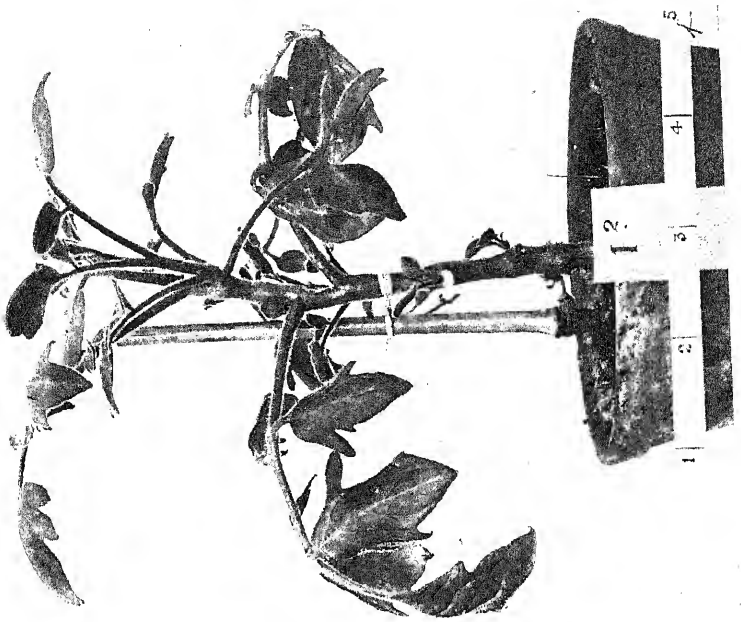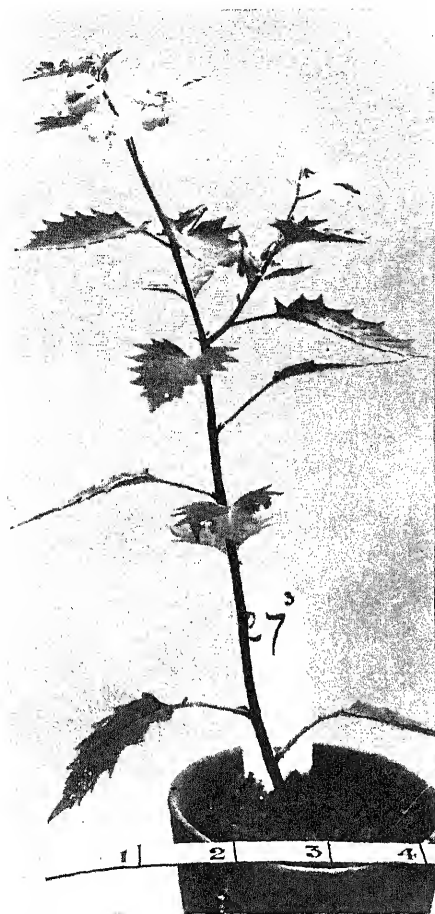
Fig. 1.

Fig. 2.

Fig. 3.
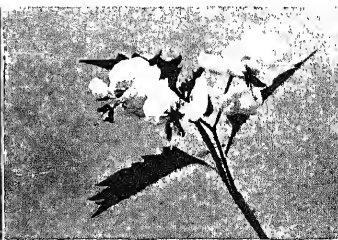
Fig. 4.

Fig. 1.
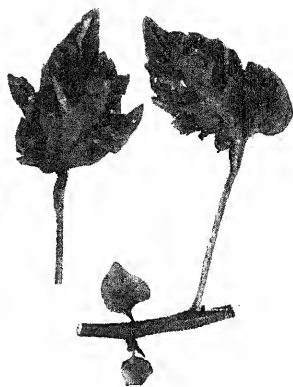
Fig. 2.

Fig. 3.

Fig. 1.

Fig. 2.

Fig. 3.
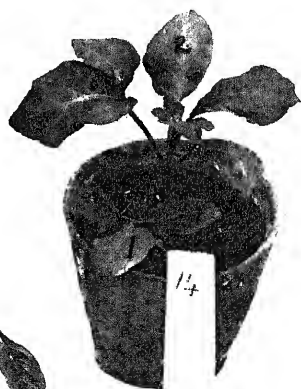
Fig. 4.

PLATE XIII.

Fig. 1. A chimaera of the combination *S. nigrum* var. *gracile-sisymbrifolium* (i).

Fig. 2. Another plant, showing pure *gracile* growth which has broken out from the top.

Figs. 3 and 4. Flowers of the chimaera, showing their peculiar structure.

PLATE XIV.

Fig. 1. Young plant of the combination *S. lycopersicum-nigrum* (i).

Fig. 2. Leaves of the same chimaera; note differences in the green colour.

Fig. 3. Adventitious shoot, at the commencement mericlinal, now developed into a
. periclinal of the type *S. lycopersicum-luteum* (ii).

PLATE XV.

Figs. 1 and 2. Plants of *S. lycopersicum-luteum* (ii). In fig. 1 the tomato core is composed
of the var. Balch's Fillbasket, and in fig. 2 of Sutton's Best of All.

Fig. 3. Transitional shoot, showing flowers and fruits.

Fig. 4. Young plant of *S. lycopersicum-guineense* (ii), the tomato core of this chimaera
is tetraploid.

PLATE XVI.

Fig. 1. Plant of *S. luteum-lycopersicum* (i).

Figs. 2 and 3. Petals and flowers of *S. lycopersicum, S. luteum, S. nigrum, S. guineense* and
of the chimaeras: *a, S. lycopersicum; b, S. nigrum* or *S. guineense; c, S. luteum; b* 1, two
layers of *S. nigrum* outside tomato; *c* 1, two layers of *S. luteum* over tomato; *b* 2, one
layer of *S. nigrum* over tomato; *c* 2, one layer of tomato outside *S. luteum*.

EXPERIMENTS WITH A PEAR-LEAFED AND FASCIATED STRAIN OF THE JAPANESE MORNING GLORY.

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(With Two Plates and Eleven Text-figures.)

CONTENTS.

INTRODUCTION.

THE phenomenon of fasciation has been observed in various groups of plants, and is generally of a temporary nature due to environmental stimulation, though sometimes hereditary. Several authors have made

experiments on this character and have obtained fairly simple results. In the Japanese morning glory, fasciation was recognised in relatively early days. The results obtained by me in breeding experiments with the fasciated strain have shown that the case is somewhat complicated by the occurrence of three or more factors affecting its manifestation. In almost all cases fasciated stems are accompanied by pear leaves, owing to the occurrence of linkage, which further complicates the genetics of the pear-leafed and fasciated plants. Some pear-leafed fasciated examples bear funnel-shaped flowers, while others bear split ones. The experiments revealed the fact that a split corolla is the effect of the "maple" factor. Consequently we may commence our account with an outline of the hereditary behaviour of pear leaf.

HEREDITARY BEHAVIOUR OF PEAR LEAF.

The pear leaf (Figs. 1, 10 and 11, Pls. XVII and XVIII, figs. 1, 8 and 9) was so named because of its resemblance to a leaf of the pear, and it is sometimes called "Kujaku[1]" leaf or "Imo[2]" leaf by our fanciers. The hereditary behaviour of this leaf was studied by Miyake and Imai (1920), and Hagiwara (1925), who recognised its simple recessive nature to the normal. Pear leaves have either double or single flowers, and the present studies were carried on with strains of the latter.

Pear leaf versus *Normal leaf*.

In crosses between normal and pear leaves the F_1 plants bear quite normal leaves. Although the pear leaf resembled in shape the cordate (= heart) leaf, which produces some recessive effect upon the hybrid leaves of cordate and normal, the F_1 leaves of the present cross showed no influence of their heterozygous nature. The F_2 generation consisted of the alternative forms in a simple ratio as indicated in Table I.

TABLE I.

The F_2 data obtained by the crossing of normal and pear leaves.

Cross	Normal leaf	Pear leaf	Total
65 × BD–B	435	139	574
L × N 1	75	26	101
Total	510	165	675
Expected	506·25	168·75	675

[1] "Kujaku" means peacock in Japanese. Pear leaves bear double flowers, in which the petaloid filaments stand out above the corollas rather like the crest of a peacock. The term was then applied to the leaves accompanying such double flowers, and eventually to pear leaves in general with no limitation in regard to their flowers.

[2] "Imo" means the Japanese yam (*Dioscorea*). The pear leaf resembles the leaf of some species of the Japanese yam in its form.

In Table II we have summarised the F_3 data, which we need not consider further as the results came out quite normally.

TABLE II.

The F_3 data of the cross 65 × BD–B.

Character of F_2	Pedigree number	Normal leaf	Pear leaf	Total
Normal leaf	$\begin{cases} 8 \\ 14 \end{cases}$	242 271	— 83	242 354
Pear leaf	7	—	125	125

Fig. 1. A fasciated seedling bearing variegated pear leaves (A 5).

The Relation of Pear and Cordate Leaves.

To understand the factorial relations between the two roundish leaves, pear and cordate (Fig. 2), I made a cross between them. The F_1 plants bore normal three-lobed leaves, being entirely different from both parents, but the leaves had roundish lobes as an effect of the recessive

cordate-leaf factor. Three families bred from such F_1 hybrids gave the results indicated in Table III.

TABLE III.

The F_2 data obtained by the crossing of cordate and pear leaves.

Cross	Normal leaf	Roundish normal leaf	Cordate leaf	Pear leaf	Total
A 1 × N 1	32	82	45	37	196
Expected	36·75	73·50	36·75	49·00	196

Fig. 2. Cordate leaf bearing normal, funnel-shaped flowers.

As the data obtained are closely in accordance with $3 : 6 : 3 : 4$, a modified ratio of dihybrid segregation, it is clear that the factors for pear and cordate leaves belong to different series of allelomorphs. Both leaves are somewhat similarly roundish in shape, but the pear leaf is rather oval and has the peculiar habit of a slight broadening in form at the connecting part of its petiole and lamina, the latter having more or less sloping shoulders. These traits enable us easily to distinguish this leaf from a cordate one. The present cross, therefore, concerns the two

factors **p**, pear leaf, and **h**, cordate leaf. The normal F_1 leaf is the combined result of two dominant factors, as far as the present cross is concerned, and the double recessive leaf retains its pear-leafed form. Under these circumstances, the expected ratio in F_2 should be:

1 PPHH + 2 PpHH	3 normal leaves
2 PPHh + 4 PpHh	6 roundish normal leaves
1 PPhh + 2 Pphh	3 cordate leaves
1 ppHH + 2 ppHh + 1 pphh	4 pear leaves

This ratio conforms fairly closely to the actual result as indicated in Table III.

The Relation of Pear and "Rangiku" leaves.

The "Rangiku" leaf (Pl. XVII, fig. 2) is represented by **i**, a recessive leaf-form factor to the normal (Imai, 1925). This factor affects the shape of cotyledons and leaves, the type of flowering, etc., in a complex manner. The cotyledons have shortened lobes, just like those of radishes, and are sometimes branched. The leaves are split irregularly into sharp-pointed lobes, and the flowers are composed of polypetalous corollas rugose in form (Pl. XVII, fig. 4). In the crosses made between "Rangiku" and pear leaves, I obtained normal-leafed F_1 hybrids reverting to their prototype, and the next generation consisted of segregating families as represented in Table IV.

TABLE IV.

The F_2 data obtained by the crossing of "Rangiku" and pear leaves.

Cross	Normal leaf	"Rangiku" leaf	Pear leaf	"Rangiku"-pear leaf	Total
M 3 × N 1	82	12	14	1	109
M 3 × N 2	142	34	21	5	202
Total	224	46	35	6	311
Expected	174·94	58·31	58·31	19·44	311

The deviation is conspicuous, though it seems to be caused by the early death of the recessive segregates. The discrepancy in the segregating ratio, which is due to the meagre production of the recessive segregates, is not rarely to be met with in the hybrid progeny of the Japanese morning glory, and the present case, therefore, is not unusual. The cross concerns the segregation of the factors, pear (**p**) and "Rangiku" (**i**) leaves. From the doubly heterozygous F_1 we should expect an F_2 segregation as follows:

1 IIPP + 2 IiPP + 2 IIPp + 4 IiPp	9 normal leaves
1 IiPP + 2 IiPp	3 "Rangiku" leaves
1 IIpp + 2 Iipp	3 pear leaves
1 iipp	1 "Rangiku"-pear leaf

Now we have a new type through the combination of two recessive factors. The specimens had narrow pear leaves, which were split, with shortened and broad petioles (Fig. 3), peculiar in shape, and somewhat small flowers with creased corollas, which are the trait of "Rangiku." The smallness of the corolla, however, is a general characteristic of the flowers of pear leaves.
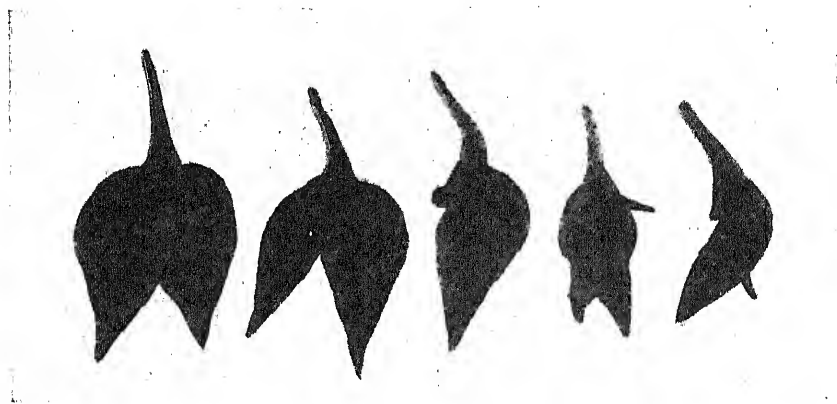
Fig. 3. Samples of "Rangiku"-pear leaves.

On the Pear-leafed strain with Split Corollas.

The pear-leafed strains used in the above cross had perfect funnel-shaped corollas, while A 5, a pure pedigree strain, had five split corollas (Pl. XVII, fig. 1). The split flowers could be identified with the maple type (Pl. XVII, fig. 6) by their relatively broad petals, and breeding tests made this clear (Imai, 1925).

The result of cross 81–1 × A 5. 81–1, one of the parents of the present cross, had normal leaves and funnel-shaped flowers. The F_1 plants bore normal leaves and flowered with perfect corollas, and gave rise to the F_2 generation shown in Table V.

TABLE V.

The F_2 data of the crossing of normal leaf and pear leaf with split corolla.

Cross	Normal leaf	Maple leaf	Pear leaf with perfect corolla	Pear leaf with split corolla	Total
81–1 × A 5	71	21	24	6	122
Expected	68·625	22·875	22·875	7·625	122

The genetic origin of the maple is attributed to A 5, which bears maple-type flowers as stated above. Hence the present segregation

concerns the factors, p and m, the latter being responsible for the maple. Theoretically, the F_2 segregation should be:

$$1\,MMPP + 2\,MmPP + 2\,MMPp + 4\,MmPp \qquad \text{9 normal leaves}$$
$$1\,mmPP + 2\,mmPp \qquad\qquad\qquad \text{3 maple leaves}$$
$$1\,MMpp + 2\,Mmpp \qquad \text{3 pear leaves with perfect corollas}$$
$$1\,mmpp \qquad\qquad \text{1 pear leaf with split corolla}$$

The pear-leafed F_2 segregates will have either perfect corollas (Pl. XVIII, fig. 8) or split ones (Pl. XVIII, fig. 9), according to their genetic composition with respect to the maple factor. Consequently the pear-leafed parent must have been a double recessive for the factors in question.

The result of crosses 22–1 × A 5 *and* 350 × A 5. The present crosses concern the relation of pear and dragon-fly leaves, the latter being transmitted as a recessive to the normal. The genetic nature of the dragon-fly leaf, however, does not seem to be simple in some cases. The F_1 plants of dragon-fly × pear had normal leaves and gave rise to the F_2 shown in Table VI.

TABLE VI.

The F$_2$ *data of the crossing of dragon-fly leaf and pear leaf with split corolla.*

Cross	Normal leaf	Dragon-fly leaf	Maple leaf	Pear leaf with perfect corolla	Elongated pear leaf with perfect corolla	Pear leaf with split corolla (contains elongated one)	Total
22–1 × A 5	43	4	14	12	1	2	76
350 × A 5	76	6	29	22	5	8	146
Total	119	10	43	34	6	10	222
Expected	93·66	31·22	41·63	31·22	10·41	13·88	222·02

The segregating number of dragon-fly leaves is distinctly below that required in theory, which may perhaps point to this character being of a more complex nature. For our present purpose however we may regard it as simple, and consider that we are dealing with the three factors of p, m and t, the last being responsible for the dragon-fly leaf. Among the pear-leafed F_2, we found some long-shaped ones, which are doubtless pear leaves carrying the dragon-fly factor. The F_2 result of the present cross (ttMMPP × TTmmpp), therefore, may be expected to be as follows:

1 TTMMPP + 2 TtMMPP + 2 TTMmPP + 2 TTMMPp + 4 TtMmPP + 4 TtMMPp + 4 TTMmPp
   + 8 TtMmPp   27 normal leaves
1 ttMMPP + 2 ttMmPP + 2 ttMMPp + 4 ttMmPp   9 dragon-fly leaves
1 TTmmPP + 2 TtmmPP + 2 TTmmPp + 4 TtmmPp + 1 ttmmPP + 2 ttmmPp   12 maple leaves
1 TTMMpp + 2 TtMMpp + 2 TTMmpp + 4 TtMmpp + 1 ttMMpp + 2 ttMmpp
                        12 pear leaves with perfect corollas (contain long-shaped pear leaves)
1 TTmmpp + 2 Ttmmpp + 1 ttmmpp
                        4 pear leaves with split corollas (contain long-shaped pear leaves)

The theoretical expectation fits the observed data fairly well provided that we do not lay stress upon the low proportion of dragon-fly leaves.

The result of crosses 320 × A 5 *and* M 3 × A 5. In these two cases a pear leaf with split corollas was crossed with a "Rangiku" leaf. Consequently the segregation may be expected to be somewhat complicated. From the normal-leafed F_1 we raised F_2, which was composed of eight phenotypes, as represented in Table VII.

TABLE VII.

The F₂ data of the crossing of "Rangiku" leaf and pear leaf with split corolla.

Cross	Normal leaf	"Rangiku" leaf	Maple leaf	Pear leaf with perfect corolla	"Mitsuo" leaf
320 × A 5	37	6	9	4	1
M 3 × A 5	102	31	44	31	9
Total	139	37	53	35	10
Expected	119·81	39·94	39·94	39·94	13·31

Cross	"Rangiku"-pear leaf with perfect corolla	Pear leaf with split corolla	"Rangiku"-pear leaf with split corolla	Total
320 × A 5	0	0	0	57
M 3 × A 5	5	4	1	227
Total	5	4	1	284
Expected	13·31	13·31	4·44	284

It is clear from the observed data that the factors now concerned are **p**, **i** and **m**, and, according to our expectation, the triple heterozygous F_1, obtained by the crossing **PPiiMM × ppIImm**, should give the F_2 members as follows:

1 IIMMPP + 2 IiMMPP + 2 IIMmPP + 2 IIMMPp + 4 IiMmPP + 4 IiMMPp + 4 IIMmPp
   + 8 IiMmPp   27 normal leaves
1 iiMMPP + 2 iiMmPP + 2 iiMMPp + 4 iiMmPp   9 "Rangiku" leaves
1 IImmPP + 2 IimmPP + 2 IImmPp + 4 IimmPp   9 maple leaves
1 IIMMpp + 2 IiMMpp + 2 IIMmpp + 4 IiMmpp   9 pear leaves with perfect corollas
1 iimmPP + 2 iimmPp   3 "Mitsuo" leaves
1 iiMMpp + 2 iiMmpp   3 "Rangiku"-pear leaves with perfect corollas
1 IImmpp + 2 Iimmpp   3 pear leaves with split corollas
1 iimmpp   1 "Rangiku"-pear leaf with split corolla

TABLE VIII.

The F₃ data of the cross M 3 × A 5, showing the segregation of leaf form.

Offspring of the normal-leafed F₂.

Number of pedigree	Normal leaf	"Rangiku" leaf	Maple leaf	Pear leaf with perfect corolla	"Mitsno" leaf	"Rangiku" pear leaf with perfect corolla	Pear leaf with split corolla	"Rangiku" pear leaf with split corolla	Total	Genetic composition
2	31	—	—	—	—	—	—	—	31	IIMMPP
Expected	31	—	—	—	—	—	—	—	31	
2	23	7	—	—	—	—	—	—	30	IIMMPP
Expected	22·5	7·5	—	—	—	—	—	—	30	
1	5	—	1	—	—	—	—	—	6	IIMmPP
Expected	4·5	—	1·5	—	—	—	—	—	6	
2	10	—	—	9	—	—	—	—	19	IIMMPp
Expected	14·25	—	—	4·75	—	—	—	—	19	
5	73	21	19	—	7	—	—	—	120	IIMmPP
Expected	67·5	22·5	22·5	—	7·5	—	—	—	120	
3	49	12	—	13	—	2	—	—	76	IIMMPp
Expected	42·75	14·25	—	14·25	—	4·75	—	—	76	
8	146	—	49	64	—	—	10	—	269	IIMmPp
Expected	151·31	—	50·44	50·44	—	—	16·81	—	269	
9	127	40	39	37	8	13	4	1	269	iiMmPp
Expected	113·48	37·83	37·83	37·83	12·61	12·61	12·61	4·20	269	

Offspring of the "Rangiku"-leafed F₂.

Number of pedigree	Normal leaf	"Rangiku" leaf	Maple leaf	Pear leaf with perfect corolla	"Mitsno" leaf	"Rangiku" pear leaf with perfect corolla	Pear leaf with split corolla	"Rangiku" pear leaf with split corolla	Total	Genetic composition
1	—	7	—	—	1	—	—	—	8	iiMmPP
Expected	—	6	—	—	2	—	—	—	8	
1	—	21	—	—	—	5	—	—	26	iiMMPp
Expected	—	19·5	—	—	—	6·5	—	—	26	
4	—	52	—	—	19	16	—	4	91	iiMmPp
Expected	—	51·19	—	—	17·06	17·06	—	5·69	91	

TABLE VIII (_continued_).

Offspring of the maple-leafed F_2.

Number of pedigree	Normal leaf	"Rangiku" leaf	Maple leaf	Pear leaf with perfect corolla	"Mitsuo" leaf	"Rangiku"-pear leaf with perfect corolla	Pear leaf with split corolla	"Rangiku" pear leaf with split corolla	Total	Genetic composition
2	—	—	36	—	—	—	—	—	36	IImmPP
Expected	—	—	36	—	—	—	—	—	36	
1	—	—	13	—	2	—	—	—	15	IimmPP
Expected	—	—	11·25	—	3·75	—	—	—	15	
2	—	—	24	—	—	—	8	—	32	IImmPp
Expected	—	—	24	—	—	—	8	—	32	
6	—	—	114	—	27	—	26	10	177	IimmPp
Expected	—	—	99·56	—	33·19	—	33·19	11·06	177	

Offspring of the pear-leafed F_2 with perfect corolla.

Number of pedigree	Normal leaf	"Rangiku" leaf	Maple leaf	Pear leaf with perfect corolla	"Mitsuo" leaf	"Rangiku"-pear leaf with perfect corolla	Pear leaf with split corolla	"Rangiku" pear leaf with split corolla	Total	Genetic composition
3	—	—	—	61	—	—	—	—	61	IIMMpp
Expected	—	—	—	61	—	—	—	—	61	
3	—	—	—	83	20	—	—	—	103	IiMMpp
Expected	—	—	—	77·25	25·75	—	—	—	103	
3	—	—	—	34	—	—	9	—	43	IIMmpp
Expected	—	—	—	32·25	—	—	10·75	—	43	
2	—	—	—	40	17	—	4	2	63	IiMmpp
Expected	—	—	—	35·44	11·81	—	11·81	3·94	63	

Offspring of the "Rangiku"-pear-leafed F_2 with perfect corolla.

Number of pedigree	Normal leaf	"Rangiku" leaf	Maple leaf	Pear leaf with perfect corolla	"Mitsuo" leaf	"Rangiku"-pear leaf with perfect corolla	Pear leaf with split corolla	"Rangiku" pear leaf with split corolla	Total	Genetic composition
1	—	—	—	—	—	1	—	—	1	iiMMpp?
Expected	—	—	—	—	—	1	—	—	1	
1	—	—	—	—	—	83	—	14	97	iiMmpp
Expected	—	—	—	—	—	72·75	—	24·25	97	

Expectation agrees in essential points with the actual data in Table VII, except for the low proportion of the combined recessive segregates. The "Mitsuo" leaves are to be regarded as a double recessive form of maple and "Rangiku." They are irregularly lobed in a peculiar way differing somewhat from "Rangiku" leaves. The term "Mitsuo," meaning "three-tailed," was derived from a variety of goldfish which has a tail so named. The flowers of this specimen are of the split "Rangiku" type (Pl. XVII, fig. 5).

An F_3 generation was raised from the F_2 of the cross M 3 × A 5, the result being summarised in Table VIII. It shows that the hypothesis put forward above covers the F_3 results.

The result of cross 326 × A 5. By hybridising A 5 with 326, which is a pure pedigree strain bearing cordate "Sasa" leaves (Fig. 4) and split flowers, we obtained normal leaves with roundish lobes in F_1. Table IX contains the F_2 data obtained from such hybrids.

TABLE IX.

The F_2 *data of the crossing of cordate "Sasa" leaf and pear leaf with split corolla.*

Cross	Normal leaf	Roundish normal leaf	Cordate leaf	Maple leaf	Cordate maple leaf
326 × A 5	74	154	76	69	13
Expected	67·39	134·79	67·39	67·39	22·46

Cross	Pear leaf with perfect corolla	Pear leaf with split corolla	Normal "Sasa" leaf	Roundish-normal "Sasa" leaf	Cordate "Sasa" leaf
326 × A 5	108	28	11	33	15
Expected	89·86	29·95	22·46	44·93	22·46

Cross	Maple-"Sasa" leaf	Cordate maple-"Sasa" leaf	Pear-"Sasa" leaf with split corolla	Pear-"Sasa" leaf with narrowly split corolla	Total
326 × A 5	14	10	28	6	639
Expected	22·46	7·49	29·95	9·98	638·96

The production of fourteen F_2 phenotypes is due to the segregation of the s_a-factor, which is responsible for the "Sasa" leaf (Imai, 1925), besides the factors of **p**, **h** and **m**, the cross producing the quadruply heterozygous F_1. The "Sasa" leaves always accompany split corollas, but the petals are a little narrower than those of the maple. The factor does not markedly change the various leaf forms, but it modifies them in a particular way into the respective "Sasa" leaves (Figs. 4, 5, 6, 7 and 8). Thus the combination of the factors for maple and "Sasa," gives narrowly split corollas divided down to the bottom of the flower

Fig. 4. A seedling of 326, bearing cordate "Sasa" leaves.

Fig. 5. A seedling bearing maple-"Sasa" leaves.
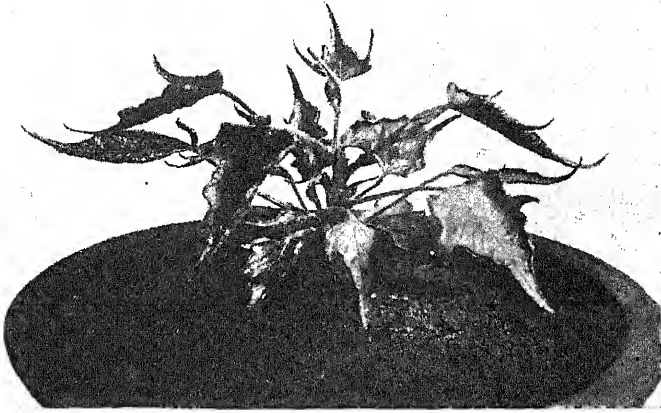
Fig. 6. A seedling bearing cordate maple-"Sasa" leaves.

Fig. 7. A seedling bearing pear-"Sasa" leaves. Fasciated!

tube (Pl. XVII, fig. 3), each factor by itself, however, resulting in split corollas with undivided tubes (Pls. XVII and XVIII, figs. 6 and 10).
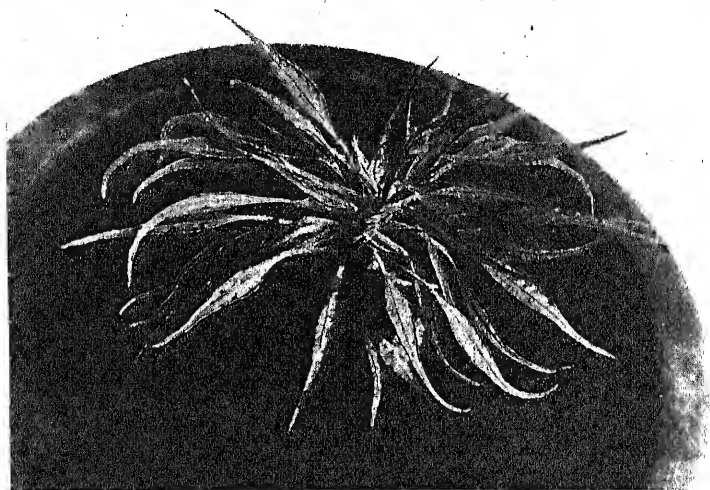
Fig. 8. A pear-"Sasa"-leafed seedling carrying maple factors. Note the slender leaves! The specimen is fasciated and the abnormality can be seen by its flattening bud. (Photographed from above.)

The genetic composition of the parents can be presumed as $s_as_a$hhMMPP and S_aS_aHHmmpp respectively. From the quadruply heterozygous F_1 we may expect the following F_2:

$1S_aS_a$HHMMPP $+ 2S_as_a$HHMMPP $+ 2S_aS_a$HHMmPP $+ 2S_aS_a$HHMMPp $+ 4S_aS_a$HHMmPP

 $+ 4S_as_a$HHMMPp $+ 4S_aS_a$HHMmPp $+ 8S_as_a$HHMmPp   27 normal leaves

$2S_aS_a$HhMMPP $+ 4S_as_a$HhMMPP $+ 4S_aS_a$HhMmPP $+ 4S_aS_a$HhMMPp $+ 8S_as_a$HhMmPP

 $+ 8S_as_a$HhMMPp $+ 8S_aS_a$HhMmPp $+ 16S_as_a$HhMmPp 54 roundish normal leaves

$1S_aS_a$hhMMPP $+ 2S_as_a$hhMMPP $+ 2S_aS_a$hhMmPP $+ 2S_aS_a$hhMMPp $+ 4S_aS_a$hhMmPP

 $+ 4S_as_a$hhMMPp $+ 4S_aS_a$hhMmPp $+ 8S_as_a$hhMmPp   27 cordate leaves

$1S_aS_a$HHmmPP $+ 2S_as_a$HHmmPP $+ 2S_aS_a$HhmmPP $+ 2S_aS_a$HHmmPp $+ 4S_as_a$HhmmPP

 $+ 4S_aS_a$HHmmPp $+ 4S_aS_a$HhmmPp $+ 8S_as_a$HhmmPp   27 maple leaves

$1S_aS_a$hhmmPP $+ 2S_as_a$hhmmPP $+ 2S_aS_a$hhmmPp $+ 4S_as_a$hhmmPp 9 cordate maple leaves

$1S_aS_a$HHMMPp $+ 2S_as_a$HHMMpp $+ 2S_aS_a$HhMMpp $+ 2S_aS_a$HHMmpp $+ 4S_as_a$HhMMpp

 $+ 4S_as_a$HHMmpp $+ 4S_aS_a$HhMmpp $+ 8S_as_a$HhMmpp $+ 1S_aS_a$hhMMpp $+ 2S_as_a$hhMMpp

 $+ 2S_aS_a$hhMmpp $+ 4S_as_a$hhMmpp   36 pear leaves with perfect corollas

$1S_aS_a$HHmmpp $+ 2S_as_a$HHmmpp $+ 2S_aS_a$Hhmmpp $+ 4S_as_a$Hhmmpp $+ 1S_aS_a$hhmmpp

 $+ 2S_as_a$hhmmpp   12 pear leaves with split corollas

$1s_as_a$HHMMPP $+ 2s_as_a$HHMMPP $+ 2s_as_a$HHMMPp $+ 4s_as_a$HHMmPP

          9 normal "Sasa" leaves

$2\,s_as_a\text{HhMMPP} + 4\,s_as_a\text{HhMmPP} + 4\,s_as_a\text{HhMMPp} + 8\,s_as_a\text{HhMmPp}$

18 roundish normal "Sasa" leaves

$1\,s_as_a\text{hhMMPP} + 2\,s_as_a\text{hhMmPP} + 2\,s_as_a\text{hhMMPp} + 4\,s_as_a\text{hhMmPp}$ 9 cordate "Sasa" leaves

$1\,s_as_a\text{HHmmPP} + 2\,s_as_a\text{HhmmPP} + 2\,s_as_a\text{HHmmPp} + 4\,s_as_a\text{HhmmPp}$

9 maple-"Sasa" leaves (Fig. 5)

$1\,s_as_a\text{hhmmPP} + 2\,s_as_a\text{hhmmPp}$ 3 cordate maple-"Sasa" leaves (Fig. 6)

$1\,s_as_a\text{HHMMpp} + 2\,s_as_a\text{HhMMpp} + 2\,s_as_a\text{HHMmpp} + 4\,s_as_a\text{HhMmpp} + 1\,s_as_a\text{hhMMpp}$

$+ 2\,s_as_a\text{hhMmpp}$ 12 pear-"Sasa" leaves with split corollas (Fig. 7, Pl. XVIII, fig. 7)

$1\,s_as_a\text{HHmmpp} + 2\,s_as_a\text{Hhmmpp} + 1\,s_as_a\text{hhmmpp}$

4 pear-"Sasa" leaves with narrowly split corollas (Fig. 8)

This expectation not only covers the F_2 data fairly well, but agrees also with the F_3 results which are collected and summarised in Table X.

BEHAVIOUR OF FASCIATION IN INHERITANCE.

An Introductory Remark.

Fasciation is a teratological character widely spread in various plant groups (see especially Masters (1869), Penzig (1890–94), Worsdell (1915), White (1916), Shirai (1925), etc.). Much literature has been published on fasciation treating of its occurrence, origin, morphology, physiology, heredity and so on. Fasciation has been universally recognised among herbs, shrubs and even trees, leading sometimes to the production of giant monstrosities. It is, however, in most cases, a transient pheno-menon, due to the stimulation of some environmental conditions or to accidental effects. As distinct from these accidental fasciations, there are records of plants exhibiting heritable fasciation, in each of which a genetical basis for the manifestation of the abnormality is involved. Some of them breed true to the abnormality in successive generations without exception, though there may be exhibited much variation in the degree of the flattening of the affected part, while others throw varying percentages of normals according to their environment or to selection. De Vries (1903) made a series of experiments with some fasciated plants.

We may cite below some of the papers dealing with the systematic analysis of fasciation by experimental crossing. The classical work of Mendel (1865) on peas includes the inheritance of fasciation or *umbellatum* character. According to him and his followers, the abnormality is transmitted as a recessive to the normal condition. East and Hayes (1911) detected a case of the simple dominance of the fasciated ear in *Zea mays*. According to the experiments made by Emerson (1912), the fasciated ear of maize was transmitted as a recessive to the normal, quite

TABLE X.

The F_3 data of the cross 326 × A 5, showing the segregation of leaf form.

Offspring of the normal-leafed F_2.

Number of pedigree N	Normal leaf	Roundish normal leaf	Cordate leaf	Maple leaf	Cordate maple leaf	Pear leaf	Normal "Sasa" leaf	Roundish normal "Sasa" leaf	Cordate "Sasa" leaf	Maple-"Sasa" leaf	Cordate maple-"Sasa" leaf	Pear-"Sasa" leaf	Total	Genetic composition
2	44	—	—	20	—	—	—	—	—	—	—	—	64	SaSaHHMmPP
Expected	48	—	—	16	—	—	—	—	—	—	—	—	64	SaSaHHMmPP
1	204	—	—	—	—	55	—	—	—	—	—	—	259	SaSaHHMMPp
Expected	194·25	—	—	—	—	64·75	—	—	—	—	—	—	259	SaSaHHMMPp
2	125	—	—	36	—	52	33	—	—	10	—	16	272	SaSaHHMmPp
Expected	114·75	—	—	38·25	—	51·00	38·25	—	—	12·75	—	17·00	272	SaSaHHMmPp

Offspring of the roundish-normal-leafed F_2.

Number of pedigree N	Normal leaf	Roundish normal leaf	Cordate leaf	Maple leaf	Cordate maple leaf	Pear leaf	Normal "Sasa" leaf	Roundish normal "Sasa" leaf	Cordate "Sasa" leaf	Maple-"Sasa" leaf	Cordate maple-"Sasa" leaf	Pear-"Sasa" leaf	Total	Genetic composition
2	42	93	53	—	—	—	—	—	—	—	—	—	188	SaSaHhMMPP
Expected	47	94	47	—	—	—	—	—	—	—	—	—	188	SaSaHhMMPP
2	4	17	6	8	3	—	—	—	—	—	—	—	38	SaSaHhMmPP
Expected	7·125	14·25	7·125	7·125	2·375	—	—	—	—	—	—	—	38	SaSaHhMmPP
5	69	174	82	63	17	62	—	—	—	—	—	—	467	SaSaHhMmPp
Expected	65·67	131·34	65·67	65·67	21·89	116·75	—	—	—	—	—	—	466·99	SaSaHhMmPp
1	32	70	28	—	—	—	5[1]	21	10	—	—	—	166	SaSaHhMMPP
Expected	31·125	62·25	31·125	—	—	—	10·375	20·75	10·375	—	—	—	166	SaSaHhMMPP
3	51	92	53	47	18	—	16	20	13	8[1]	1	—	319	SaSaHhMmPP
Expected	44·86	89·72	44·86	44·86	14·95	—	14·95	29·91	14·95	14·95	4·98	—	318·99	SaSaHhMmPP
5	45	104	50	56	12	80	19[1]	21	22	13	3	25	450	SaSaHhMmPp
Expected	47·46	94·92	47·46	47·46	15·82	84·38	15·82	31·64	15·82	15·82	5·27	28·13	450	SaSaHhMmPp

Offspring of the cordate-leafed F_2.

							Total	Genotype
1	—	—	—	—	—	—	1	SaSahhMMPP?
Expected	—	—	—	—	—	—	1	
1	—	—	—	—	—	5	16	SaSahhMmPP
Expected	—	—	—	—	—	4	16	
2	11	—	—	—	34	—	106	SaSahhMMPp
Expected	12	—	—	—	26·5	—	106	
1	72	—	—	—	1	1	3	SaSahhMmPp?
Expected	79·5	—	—	—	0·75	0·56	3	
2	1	—	—	51(1)	—	26	207	SaSahhMmPP
Expected	1·69	—	—	38·81	—	38·81	12·94 / 8 / 207	
3	122	—	—	32	54	21	279	SaSahhMmPp
Expected	116·44	—	—	39·23	52·29	39·23	13·08 / 11 / 17·43 / 17 / 278·96	
	144							
	117·70							

Offspring of the maple-leafed F_2.

							Total	Genotype
1	—	49	12	15	—	—	76	SaSaHhmmPp
Expected	—	42·75	14·25	19·00	—	—	76	
1	—	18	—	—	7	—	25	SaSaHHmmPP
Expected	—	18·75	—	—	6·25	—	25	
1	—	42	20	—	15	6	83	SaSaHhmmPP
Expected	—	46·69	15·56	—	15·56	5·19	83	
1	—	97	—	30	30	—	12(1) / 169	SaSaHHmmPp
Expected	—	95·06	—	31·69	31·69	—	10·56 / 169	
2	—	44	11	25	15	1	9 / 105	SaSaHHmmPp
Expected	—	44·30	14·77	19·69	14·77	4·92	6·56 / 105	

Offspring of the cordate-maple-leafed F_2.

				Total	Genotype
1	—	—	9	4 / 13	SaSahhmmPP
Expected	—	—	9·75	3·25 / 13	

TABLE X (continued).

Offspring of the pear-leafed F_2 with perfect corolla.

Number of pedigree	Pear leaf with perfect corolla	Pear leaf with split corolla	Pear-"Sasa" leaf with entire corolla	Pear-"Sasa" leaf with narrowly split corolla	Total	Genetic composition
6	85	—	—	—	85	} $S_aS_a(?)$MMpp
Expected	85	—	—	—	85	
2	7	3	—	—	10	} $S_aS_a(?)$Mmpp
Expected	7·5	2·5	—	—	10	
3	25	4	8	1	38	} $S_aS_a(?)$Mmpp
Expected	21·375	7·125	7·125	2·375	38	

Offspring of the pear-leafed F_2 with split corolla.

2	—	10	—	—	10	} $S_aS_a(?)$mmpp
Expected	—	10	—	—	10	
1	—	15	—	6	21	} $S_aS_a(?)$mmpp
Expected	—	15·75	—	5·25	21	

Offspring of the normal-"Sasa"-leafed F_2.

Number of pedigree	Normal "Sasa" leaf	Maple-"Sasa" leaf	Pear-"Sasa" leaf with split corolla	Pear-"Sasa" leaf with narrowly split corolla	Total	Genetic composition
2	10	—	—	—	10	} s_as_aHHMMPP
Expected	10	—	—	—	10	
1	3	1	—	—	4	} s_as_aHHMmPP
Expected	3	1	—	—	4	

Offspring of the cordate-"Sasa"-leafed F_2.

Number of pedigree	Cordate "Sasa" leaf	Cordate maple-"Sasa" leaf	Pear-"Sasa" leaf with split corolla	Pear-"Sasa" leaf with narrowly split corolla	Total	Genetic composition
1	2	—	—	—	2	} $s_as_a$hhMMPP ?
Expected	2	—	—	—	2	
1	2	1	2	0	5	} $s_as_a$hhMmPp
Expected	2·81	0·94	0·94	0·31	5	

N.B. Some records of the flower type of the pear-leafed segregates may be incomplete and doubtful cases are not considered in classification.

The numeral in brackets represents the number of the plants which made somatic variation to a non-"Sasa" condition.

contrary to the one observed by the former investigators. In some strains, however, the hereditary behaviour of fasciation was not simple, and he thought that two factors were probably involved. White (1916) studied the inheritance of a fasciated *Nicotiana* by crossing it with its normal prototype and found it to be of a simple Mendelian type, the heterozygotes being intermediate in form. The result was not so simple, however, when the fasciated strain was crossed with the normals of

different varieties. According to White, such complexity is due to a difference in the "genotypical environments." Nagai (1926) in his recently published data on the inheritance of soy-beans worked with fasciation, and concluded that it was of a simple recessive nature.

In the Japanese morning glory fasciated specimens have long been recognised. Now we find the strains either breeding true or throwing some normals. The oldest figure of a fasciated specimen of this plant is

Fig. 9. A fasciated specimen illustrated in an old book, *Asagao-Sô*, 1817.

the one given in *Kadan-Asagao-Tsû* (1815), which was published over one hundred and ten years ago. The attractive illustrations of this monstrosity (Fig. 9) are found in various other old books. According to these old authors, the heritability of fasciation was not strong in their days, but it was sometimes considered to be a temporary expression of the normals due to a "disease" or "supernutrition." We may conclude, therefore, that strains breeding true to the character are of relatively recent origin.

Yamaguchi (1916) studied the fasciation of the Japanese morning

20–2

glory from a morphological and physiological point of view, but his paper contains no genetic analysis. Hagiwara (1924, 1926) published his genetic results on the fasciated morning glory and assumed two recessive factors for an abnormal manifestation. His data, however, did not contain sufficient individuals to solve such a complex problem.

My experiments on fasciation were started in 1921 and the hybrid generations ran to F_4 in one cross, including a very extensive cultivation of F_3. My conclusion is so complex that it contains some points incompatible with that which was drawn by Hagiwara.

The Results of Experiments.

The fasciated pedigree strain used as one of the parents in my breeding experiments was A 5, a pear leaf with a split corolla (Fig. 1, Pl. XVII, fig. 1). The strain has bred true to the type for generations on self-fertilisation, and the progeny always consisted of specimens having distinctly flattened stems. By crossing this strain with normals we raised the F_1 plants, which are quite normal, representing the dominancy of normality. In the F_2 generation, the fasciated segregates were relatively very few as is indicated in Table XI.

TABLE XI.

The F_2 data showing the segregation of fasciation and the related character.

Class	Cross	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
A	326 × A 5	467	153	2	17	639	2·97
	81–1 × A 5	92	27	—	3	122	2·46
Total		559	180	2	20	761	2·89
B	320 × A 5	53	3	—	1	57	1·75
	350 × A 5	111	34	—	1	146	0·68
	M 3 × A 5	186	40	—	1	227	0·44
	22–1 × A 5	61	15	—	—	76	0·00
Total		411	92	—	3	506	0·59
Grand total		970	272	2	23	1267	1·97

In the grand total, the fasciated stems form only 1·97 per cent. The proportion in which fasciated plants appear varies considerably in different crosses, though, roughly speaking, it may be classified into two categories, viz. a relatively high proportion (class A) and a relatively low one (class B). In class A, two crosses, 326 × A 5 and 81–1 × A 5, gave fasciated plants in the proportion of 2·97 per cent. and 2·46 per cent. respectively, the average being 2·89 per cent. In the other crosses,

class B, three matings, 320 × A 5, 350 × A 5 and M 3 × A 5, gave only one fasciated individual in each F_2 progeny, which numbered respectively 57, 146 and 227. The remaining cross 22–1 × A 5 segregated no fasciated specimen among 76 F_2 offspring. The average ratio of the fasciated stems in class B was only 0·59 per cent.

An analysis of class A. With the cross 326 × A 5 I made a genetic analysis of fasciation on a comparatively extensive scale. The proportion

TABLE XII.

The F_3 data of the cross 326 × A 5, *showing the segregation of fasciation and the related character.*

Offspring of the normal stemmed and non-pear-leafed F_2.

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
Total of 19 pedigrees	1282	—	—	—	1282	—
Total of 8 pedigrees	324	106	—	—	430	—
43	24	—	1	—	25	4·00
1	205	41	—	13	259	5·02
7	128	32	—	9	169	5·33
10	115	31	—	8	154	5·19
11	7	—	—	1	8	12·50
12	95	6	1	12	114	11·40
28	29	12	—	9	50	18·00
32	12	—	—	1	13	7·69
33	51	10	—	8	69	11·59
34	167	42	—	9	218	4·13
36	40	14	—	4	58	6·90
40	128	38	1	10	177	6·21
42	41	6	—	2	49	4·08
46	74	17	1	3	95	4·21
49	43	1	—	12	56	21·43
54	63	23	—	8	94	8·51
57	3	1	—	1	5	20·00
Total	1201	174	3	110	1588	7·12

Offspring of the normal stemmed and pear-leafed F_2.

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
Total of 5 pedigrees	—	34	—	—	34	—
4	—	41	—	6	47	12·77
6	—	6	—	2	8	25·00
16	—	15	—	3	18	16·67
17	—	5	—	1	6	16·67
19	—	13	—	2	15	13·37
29	—	9	—	2	11	18·18
35	—	8	—	3	11	27·27
38	—	6	—	2	8	25·00
41	—	11	—	1	12	8·33
55	—	5	—	2	7	28·57
Total	—	119	—	24	143	16·78

TABLE XIII.

The other F₃ data of the cross 326 × A 5, showing the segregation of fasciation and the related character.

Offspring of the normal-stemmed and non-pear-leafed F_2.

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
Total of 30 pedigrees	2167	—	—	—	2167	—
Total of 17 pedigrees	791	226	—	—	1017	—
90	29	—	1	—	30	3·33
111	2	—	1	—	3	33·33
128	244	—	1	—	245	0·41
Total	275	—	3	—	278	1·08
60	21	—	—	5	26	19·23
63	115	24	—	1	140	0·71
68	16	2	—	1	19	5·26
72	115	24	—	6	145	4·14
77	47	7	1	10	65	16·92
78	40	11	—	1	52	1·92
79	78	4	—	24	106	22·64
82	56	8	—	12	76	15·79
83	77	2	1	17	97	18·56
86	36	1	1	14	52	28·85
97	43	13	—	2	58	3·45
98	44	15	—	6	65	9·23
99	144	13	—	33	190	17·37
101	17	—	—	4	21	19·05
103	65	6	1	25	97	26·80
104	5	1	—	1	7	14·29
105	97	22	—	5	124	4·03
106	31	8	—	4	43	9·30
112	108	28	—	7	143	4·90
113	96	28	—	11	135	8·15
116	67	17	—	4	88	4·55
117	84	20	—	4	108	3·70
118	116	15	—	19	150	12·67
120	12	2	—	1	15	6·67
121	25	5	—	3	33	9·09
122	113	28	—	4	145	2·76
126	59	18	—	3	80	3·75
127	72	16	—	4	92	4·35
129	70	2	—	26	98	26·53
132	15	5	—	2	22	9·09
133	98	3	1	21	123	17·89
136	47	14	—	8	69	11·59
137	43	10	—	3	56	5·36
138	79	16	—	10	105	9·52
139	79	29	—	10	118	8·47
142	73	19	—	3	95	3·16
146	17	—	1	3	21	19·05
148	22	10	—	1	33	3·03
152	72	20	—	7	99	7·07
158	57	17	—	18	92	19·57
159	175	28	—	13	216	6·02
160	145	34	—	8	187	4·28
173	52	2	—	7	61	11·48
176	86	—	2	26	114	24·56
177	77	23	—	6	106	5·66
178	27	—	—	8	35	22·86
179	59	14	—	3	76	3·95
180	119	27	—	8	154	5·19
181	31	8	—	5	44	11·36
Total	3242	619	8	427	4296	10·13

TABLE XIII (*continued*).

Offspring of the normal-stemmed and pear-leafed F_2.

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
Total of 7 pedigrees	—	56	—	—	56	—
64	—	7	—	1	8	12·50
73	—	14	—	4	18	22·22
93	—	3	—	1	4	25·00
95	—	14	—	3	17	17·65
96	—	10	—	3	13	23·08
123	—	31	—	7	38	18·42
131	—	6	—	2	8	25·00
145	—	13	—	1	14	7·14
151	—	10	—	3	13	23·08
161	—	10	—	1	11	9·09
165	—	8	—	2	10	20·00
Total	—	126	—	28	154	18·18

Offspring of the fasciated and pear-leafed F_2, including false normals.

	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
110	—	—	—	17	17	100·00
144	—	1	—	25	26	96·15
155	—	—	—	2	2	100·00
162	—	1	—	2	3	66·67
169*	—	1	—	9	10	90·00
182*	—	—	—	2	2	100·00
183	—	3	—	2	5	40·00
Total	—	6	—	59	65	90·77

The asterisked pedigrees are the progenies of false normals.

of the fasciated stems in F_2 from this cross was 2·97 per cent., which stands between the recessive ratios of 6·25 per cent. of a dihybrid polymery and 1·56 per cent. of a trihybrid one. In F_3 the segregating proportion varies considerably as indicated in Table XII. Table XIII contains the data of the other F_3, the record of which was taken from the bed when the seedlings were about one foot high. If we make a variation table in regard to the segregating proportion of the fasciateds in each F_3 pedigree, the result will be as represented in Table XIV, and
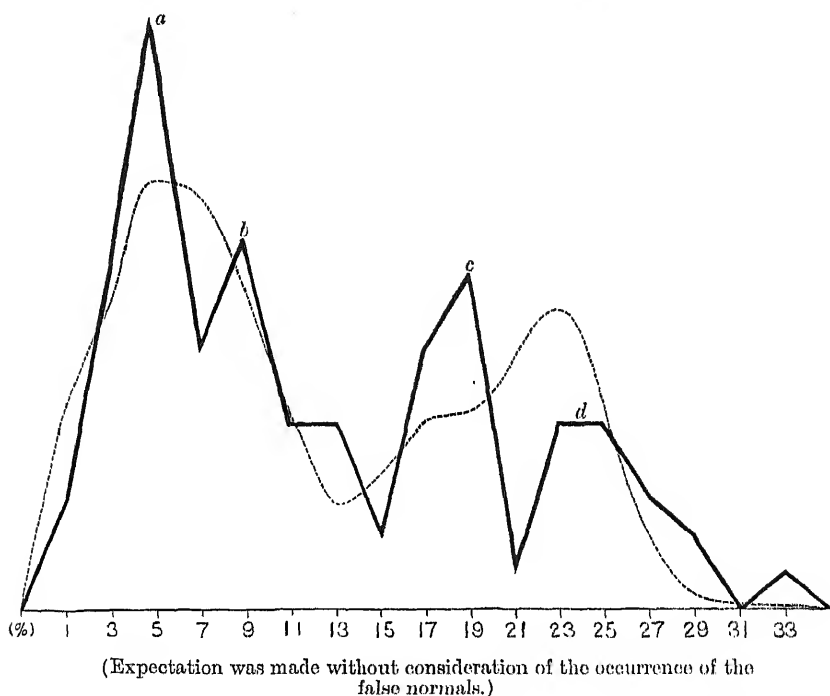
TABLE XIV.

Variation table of the frequency in the segregating proportion of fasciation.

Segregating percentage	1	3	5	7	9	11	13	15	17	19	21
From Table XII	—	1	6	3	2	2	3	—	3	2	1
From Table XIII	3	8	10	4	8	3	2	2	4	7	—
Total	3	9	16	7	10	5	5	2	7	9	1

Segregating percentage	23	25	27	29	31	33	Total	Average proportion
From Table XII	—	2	1	1	—	—	27	12·93 %
From Table XIII	5	3	2	1	—	1	63	12·27 %
Total	5	5	3	2	—	1	90	12·47 %

TABLE XV.

*The variation curves of the observed and theoretical frequency distributions
in the proportion of the fasciated segregates in the* F_3 *pedigrees.*

(Expectation was made without consideration of the occurrence of the
false normals.)

Thick line.........Observed frequency   Dotted line.........Theoretical frequency

the curve represented in Table XV by a thick line is drawn on the basis
of these figures. The frequency curve in the diagram agrees with no
simply segregating result, as it contains several high and low modes.
Of these, the mode *d* is sure to have been made up by a body of the
monohybrid segregating families, and the result must be accounted for
by presuming more than two factors constituting the non-cumulative
polymery in inheritance. An analysis of the data shows that the case
concerns three factors, and that the triple recessives are the fasciated
stems. If we represent these factors by f^1, f^2 and f^3, the fasciated parent
of the cross is to be regarded as $f^1f^1f^2f^2f^3f^3$, and its partner as a trebly
dominant homozygote. From the trebly heterozygous F_1 we should
have the following F_2 segregates:

$1 F^1 F^1 F^2 F^2 F^3 F^3 + 2 F^1 f^1 F^2 F^2 F^3 F^3 + 2 F^1 F^1 F^2 f^2 F^3 F^3 + 2 F^1 F^1 F^2 F^2 F^3 f^3 + 4 F^1 f^1 F^2 f^2 F^3 F^3$

$+ 4 F^1 f^1 F^2 F^2 F^3 f^3 + 4 F^1 F^1 F^2 f^2 F^3 f^3 + 8 F^1 f^1 F^2 f^2 F^3 f^3 + 1 f^1 f^1 F^2 F^2 F^3 F^3 + 2 f^1 f^1 F^2 f^2 F^3 F^3$

$+ 2 f^1 f^1 F^2 F^2 F^3 f^3 + 4 f^1 f^1 F^2 f^2 F^3 f^3 + 1 F^1 F^1 f^2 f^2 F^3 F^3 + 2 F^1 f^1 f^2 f^2 F^3 F^3 + 2 F^1 F^1 f^2 f^2 F^3 f^3 + 4 F^1 f^1 f^2 f^2 F^3 f^3$

$+ 1 F^1 F^1 F^2 F^2 f^3 f^3 + 2 F^1 f^1 F^2 F^2 f^3 f^3 + 2 F^1 F^1 F^2 f^2 f^3 f^3 + 4 F^1 f^1 F^2 f^2 f^3 f^3 + 1 f^1 f^1 f^2 f^2 F^3 F^3 + 2 f^1 f^1 f^2 f^2 F^3 f^3$

$+ 1 f^1 f^1 F^2 F^2 f^3 f^3 + 2 f^1 f^1 F^2 f^2 f^3 f^3 + 1 F^1 F^1 f^2 f^2 f^3 f^3 + 2 F^1 f^1 f^2 f^2 f^3 f^3$ 63 normal stems

$1 f^1 f^1 f^2 f^2 f^3 f^3$ 1 fasciated stem

In such a trihybrid, the theoretical proportion of the fasciateds is only 1·56 per cent., about half as much as that of the actual case (2·98 per cent.!). How can this difference be accounted for? According to my opinion, this can be explained as a result of linkage between two fasciation factors. In attempting to estimate the precise behaviour of the factorial relation, we meet with a serious difficulty in the occurrence of *false normals* as fluctuations from among the fasciateds. Among the fasciated F_2, those which produced some seeds and gave an opportunity to test their offspring are Nos. 110, 144, 155, 162 and 183, five in number. Some of them gave only fasciated progeny, while the others threw some normals. Hagiwara (1926) regarded such normals as mutants without observing their offspring. In my view, however, they are to be attributed to fluctuations in the manifestation of fasciation. If such is the case we should have a result similar to that from the false normals which appeared in these pedigrees. Two normals obtained in F_3 of Nos. 144 and 162 were prepared for such a test. On selfing, they gave 2 normals and 10 fasciateds in one case and in the other 3 fasciateds only in F_4. This result agrees with the data observed in the progeny of certain fasciated segregates. No one could have told that these F_4 plants were the progeny of a false F_3 normal without a record of the preceding generation. Nos. 169 and 182 were both F_2 plants having quite normal stems, but for the most part their progeny consisted of fasciated segregates as shown in Table XIII. The result they gave is precisely similar to that from the five fasciated F_2 plants cited above. With such evidence, we can safely conclude that the normals which appeared among the progeny of the fasciated families were due to a false manifestation induced by fluctuation, or, in other words, plants which are genotypically fasciated sometimes remain normal throughout their growth. The degree of flattening of the stem in the parental fasciated pedigree strain, A 5, is very evident (Fig. 1) and it always breeds true to the type, throwing no false normals. The fasciated specimens found in F_2, F_3 and F_4, however, differed from one another in their degree of flattening, varying through all gradations (Figs. 10 and 11, Pl. XVIII, figs. 8 and 9). In the least fasciated individuals, the flattening occurs only in a portion of the stem, most of the parts remaining quite normal. Such plants may be recorded as normals in

Fig. 10. A fasciated segregate bearing pear leaves. Pear leaves sometimes are lobed nearly like roundish normal leaves.

Fig. 11. Weakly fasciated pear leaf. Note the disordered phyllotaxy!

an early census, but they reveal themselves as fasciateds on later observation. Hagiwara (1926) observed a specimen, on which only a branch was fasciated, and he regarded this as due to a bud-variation. Plants of such a nature, however, were of not infrequent occurrence in my experiments, and no difference between the progenies of the fasciated and normal parts could be detected by examining their inheritance. So it appears to be a little premature to attribute such a case to bud-variation.

On the evidence cited above, we must recognise the occurrence of false normals in the hybrid progeny of the present cross. This would affect the ratio of the fasciated segregates, and the deficit in their expected numbers may be roughly estimated from the data recorded on the progeny of the fasciateds and false normals. From these data we must reckon 8 false normals to 66 fasciateds in 74 plants, a deficit among the fasciateds of 10·81 per cent. The proportion may be calculated in another way, *i.e.* from the ratio of the false normals to the fasciateds in F_2. The false normals detected in F_2, however, were only 2 in number, from which meagre figure we cannot expect to obtain a more precise value than the former case. The deficit of 10·81 per cent., however, was calculated not only on the basis of an insufficient number, but it contains also some unfavourable points. The occurrence of false normals shows that the environment had some effect upon the manifestation of fasciation. But, as already pointed out, the parental pedigree strain, A 5, always breeds true to its type. Hence the determination of the fasciated character in this pedigree strain is so perfect that the environment cannot affect its manifestation, though the fasciation may vary somewhat in degree. Why then does the manifestation fluctuate in the hybrid progeny, and not in the parental pedigree strain? The inconstancy of fasciation in its inheritance was in early days a general phenomenon in the cultivation of the Japanese morning glory. In my opinion, the difference is due to the occurrence of a modifier or modifiers, which affect the degree and production of fasciation in its manifestation. The old fasciation accompanied such a factor or factors in their fluctuating representation, while in some strains, which we now have, these factors have slipped out. In the present cross, they came from the normal parent, and segregation in respect of them takes place in the hybrid progeny. It would be a troublesome business to determine the precise behaviour of such a modifier or modifiers, and the solution of this problem will be attempted in my future experiments. The data, on which the value of the deficit was estimated, therefore, must contain something

impure in them, so that its value must be regarded as representing only an approximate figure.

If we neglect the deficit of fasciated plants the value of crossing over between two fasciation factors may be estimated by the formula,

$$\frac{x^2}{16x^2 + 32x + 16} = \frac{19}{639}.$$

From this formula the gametic ratio is determined to be $2 \cdot 22 : 1$. If we assume the fasciation factors that are linked together to be f^1 and f^2, about 31 per cent. of crossing over takes place between them. A tentative application of the $10 \cdot 81$ per cent. deficit in this calculation will change the figure of 19 fasciateds into $19 \div (100\% - 10 \cdot 81\%) = ca.\ 21$ fasciateds, including the false normals. On this view, a more precise gametic ratio is estimated to be $2 \cdot 64 : 1$ or $ca.\ 3 : 1$, or about 20–25 per cent. of crossing over. If we calculate the F_2 expectation on the basis of a $3 : 1$ gametic ratio, the result is as shown in Table XVI.

TABLE XVI.

The theoretical F_2 in the segregation of fasciation.

Genotype	Its ratio Formula	Value ($x=3$)	Phenotype	Its ratio Formula	Value ($x=3$)
$F^1F^1F^2F^2F^3F^3$	x^2	9			
$F^1F^1F^2F^2F^3f^3$	$2x^2$	18			
$F^1F^1F^2f^2F^3F^3$	$2x$	6			
$F^1f^1F^2F^2F^3F^3$	$2x$	6			
$F^1F^1F^2F^2f^3f^3$	x^2	9			
$F^1F^1f^2f^2F^3F^3$	1	1			
$f^1f^1F^2F^2F^3F^3$	1	1			
$F^1F^1F^2f^2F^3f^3$	$4x$	12			
$F^1f^1F^2F^2F^3f^3$	$4x$	12			
$F^1F^1F^2f^2f^3F^3$	$2x^2+2$	20			
$F^1f^1F^2f^2F^3f^3$	$4x^2+4$	40			
$F^1F^1f^2f^2F^3f^3$	$2x$	6			
$F^1f^1F^2f^2f^3f^3$	$2x$	6			
$F^1F^1f^2f^2F^3f^3$	2	2	Normal	$15x^2+32x+16$	247
$F^1f^1f^2f^2F^3F^3$	$2x$	6			
$f^1f^1F^2F^2F^3f^3$	2	2			
$f^1f^1F^2f^2F^3F^3$	$2x$	6			
$F^1f^1F^2f^2f^3f^3$	$2x^2+2$	20			
$F^1f^1f^2f^2F^3f^3$	$4x$	12			
$f^1f^1F^2f^2F^3f^3$	$4x$	12			
$F^1F^1f^2f^2f^3f^3$	1	1			
$F^1f^1f^2f^2f^3f^3$	$2x$	6			
$f^1f^1F^2f^2f^3f^3$	1	1			
$f^1f^1F^2f^2f^3f^3$	$2x$	6			
$f^1f^1f^2f^2F^3F^3$	x^2	9			
$f^1f^1f^2f^2F^3f^3$	$2x^2$	18			
$f^1f^1f^2f^2f^3f^3$	x^2	9	Fasciated	x^2	9
Total	$16x^2+32x+16$	256		$16x^2+32x+16$	256

As discussed above, the F_2 data may be fairly accounted for by the hypothesis suggested by me, though its confirmation must depend upon the data of succeeding generations. We may now test it with the F_3 results.

Part of the F_3 plants were sown in the beginning of May and the seedlings were later transplanted into a field where the plants were allowed full growth. The data from these plants are collected in Table XII. But as my means were limited I was unable to raise a sufficient number of plants in this way. For further data I made seed-bed observations on the remaining F_3 seeds sown in early June. Fortunately the weather was reasonably fair, and relatively few seedlings were damaged in the crowded culture. A record of them was taken when they were about one foot in height, as summarised in Table XIII. Owing to doubts whether cases of weak fasciation would be evident in early development, I feared that some fasciated specimens might have been missed in this record, because the census was taken before the attainment of full growth. However, this fear proved to be almost groundless, at least in the present case, for the average segregating proportion of the fasciateds was 12·93 per cent. in the normally full-grown culture and 12·27 per cent. in the mass culture, representing practically the same result. This fact may admit of a discussion of both sets of data together. The variation curve of the segregating frequency of such combined F_3 pedigrees is polymodal, as indicated by a thick line in Table XV, in which we may point out roughly four definite modes, a, b, c and d. The expected segregating types of the F_3 pedigrees are calculated in Table XVII, on the basis of $3 : 1$ gametic ratio in the linkage between $\mathbf{F}^1$ and $\mathbf{F}^2$.

TABLE XVII.

The theoretical segregating types of F_3 *in the segregation of fasciation.*

Genetic composition	Linkage	Ratio	Normal versus fasciation	Formula	% of fasciation	Segregating type
$F^1f^1F^2f^2F^3f^3$	Coupling	36	247 : 9	$15x^2 + 32x + 16 : x^2$	3·52	III
	Repulsion	4	255 : 1	$16x^2 + 32x + 15 : 1$	0·39	I
$F^1f^1F^2f^2f^3f^3$	Coupling	18	55 : 9	$3x^2 + 8x + 4 : x^2$	14·06	V
	Repulsion	2	63 : 1	$4x^2 + 8x + 3 : 1$	1·56	II
$F^1f^1f^2f^2F^3f^3$		12				
$f^1f^1F^2f^2F^3f^3$		12	15 : 1	15 : 1	6·25	IV
$F^1f^1f^2f^2f^3f^3$		6				
$f^1f^1F^2f^2f^3f^3$		6	3 : 1	3 : 1	25·00	VI
$f^1f^1f^2f^2F^3f^3$		18				

Thus we expect six segregating types. The theoretical number and its average value of each segregating type applied to the total observed

are represented at the bottom lines of Table XVIII. In an attempt to make a curve analysis on the basis of these figures, we obtained a variation distribution of the segregating types as indicated in the body of the table.

TABLE XVIII.

Variation table of the frequency distribution in the segregating types of fasciation in F_3.

%	\multicolumn{6}{c}{Segregating type}	Total (theoretical)	Observed
	I	II	III	IV	V	VI		
1	2·105	0·539	2·856	0·027	—	—	5·518	3
3	0·742	0·523	6·677	0·286	—	—	8·228	9
5	0·265	0·371	9·414	1·590	—	—	11·640	16
7	0·048	0·133	6·677	4·451	0·001	—	11·310	7
9	0·004	0·024	2·386	6·276	0·018	—	8·708	10
11	—	0·002	0·429	4·451	0·215	—	5·097	5
13	—	—	0·039	1·590	1·193	0·001	2·823	5
15	—	—	0·002	0·286	3·338	0·032	3·658	2
17	—	—	—	0·026	4·707	0·358	5·091	7
19	—	—	—	0·001	3·338	1·988	5·327	9
21	—	—	—	—	1·193	5·564	6·757	1
23	—	—	—	—	0·215	7·845	8·060	5
25	—	—	—	—	0·018	5·564	5·582	5
27	—	—	—	—	0·001	1·988	1·989	3
29	—	—	—	—	—	0·358	0·358	2
31	—	—	—	—	—	0·032	0·032	—
33	—	—	—	—	—	0·001	0·001	1*
Total (theoretical)	3·158	1·579	28·421	18·947	14·211	23·684	90·000	90
Average	0·526	2·210	4·868	9·526	16·632	23·264	12·466	12·466

$\sigma = 4\cdot079$, in the total variation.
$\sigma = 1\cdot207$, in the variation for each segregating type.
$\chi^2 = 24\cdot575.$ $P = 0\cdot078.$

* This was neglected in the calculation of χ^2, because its progeny consisted of only three individuals.

The sum of these six variation numbers, as given under the column of "Total," is not very different from that of the number observed as represented in the next column, the value of P being 0·078. The figures thus theoretically estimated were drawn in a curve with a dotted line in Table XV, in which a comparison is made with the observed frequency. Neither of two curves entirely overlaps the other, but we can see a rough resemblance between them. On comparing the two curves, the mode c of the thick line stands out beyond the dotted line. The larger part of this peak may be expected to be composed of the families of segregating type V, and suggests a somewhat more intense linkage between f^1 and f^2. The hypothesis offered above seems to be verified by the F_3 results, though the calculation here attempted was made in ignorance of the occasional failure of fasciated plants to manifest their fasciation, which

may frequently occur in the hybrid progeny. The deficit in fasciateds should vary with the segregation of the modifiers if more than two of them entered into this cross. Under this circumstance, the prediction of the exact number expected is a difficult matter.

On my view the expected ratio of homogenous and segregating families is 114 to 133, and we shall see how this expectation is realised in the observed data. Of 183 non-fasciated F_3 families, 120 contained over twenty individuals each. The composition of these families is indicated in Table XIX, those containing but few progeny being omitted.

TABLE XIX.

A genotypic comparison of F_2 *for the segregation of fasciation.*

	True-breeding	Segregating	Total
Observed	56	64	120
Expected	55·38	64·62	120

The number theoretically calculated on the basis of 114 : 133 thus accords fairly well with the actual data.

The genetic behaviour of fasciation reveals the complexity of the problem even more when the study is extended to other crosses, and I have found a more complicated case in another cross, the data of which are represented in the next section.

An analysis of class B. The results given in Table XI indicate roughly two types of segregation, viz. a relatively higher and a relatively lower proportion in the production of the fasciated segregates, the average proportion in the latter class, B, being only 0·59 per cent., or only about one-fifth of that of the former class, A. This lower proportion is not a matter of accidental occurrence, but depends upon a genetic difference. That this is not a speculative suggestion is evident from an inspection of the data collected in Table XX containing the F_3 results of the cross M 3 × A 5, a cross of class B. The data, however, do not contain enough individuals for attempting a curve analysis. In the calculation of fasciation in the total normal F_3 progenies, 5·75 per cent. is the result of the cross 326 × A 5, while that of cross M 3 × A 5 is only 3·54 per cent. We raised the offspring of two F_2 fasciateds, of which one (No. 23) produced three specimens with flattened stems, while the other (No. 34) gave sixteen offspring of which seven were false normals. From such evidence, the difference may be regarded genetically either as due to the occurrence of an additional fasciation factor, or of a modifier or modifiers. Hence segregation in the hybrid progeny of the present cross

TABLE XX.

The F_2 *data of the cross* M 3 × A 5, *showing the segregation of fasciation and the related character.*

Offspring of the normal-stemmed and non-pear-leafed F_2.

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
Total of 13 pedigrees	218	—	—	—	218	—
Total of 29 pedigrees	643	169	—	—	812	—
15	15	5	—	2	22	9·09
16	17	4	—	3	24	12·50
27	19	11	—	1	31	3·23
45	15	2	—	3	20	15·00
46	32	8	—	1	41	2·44
58	10	5	—	1	16	6·25
62	12	7	—	2	21	9·52
Total	120	42	—	13	175	7·43

Offspring of the normal-stemmed and pear-leafed F_2.

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
Total of 3 pedigrees	—	26	—	—	26	—
8	—	41	—	9	50	18·00
12	—	7	—	1	8	12·50
18	—	26	—	4	30	13·33
31	—	41	—	8	49	16·33
37	—	32	—	5	37	13·51
44	—	26	—	6	32	18·75
48	—	43	—	1	44	2·27
53	—	44	—	3	47	6·38
63	—	21	—	5	26	19·23
Total	—	281	—	42	323	13·00

Offspring of the fasciated and pear-leafed F_2.

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total	% of fasciated stems
23	—	—	—	3	3	100·00
34	—	7	—	9	16	56·25
Total	—	7	—	12	19	63·16

should be more complicated than that of the cross 326 × A 5. The proportions of non-fasciated families which breed true to normal, and those which segregate fasciateds, should be somewhat different from the proportions observed in the previous cross; for some increase may be expected in the proportion of the former sort of families. Actually we have 26 families of the former and 14 of the latter, omitting those containing less than twenty individuals. The proportion of segregating families in the total is 35 per cent., and 53·33 per cent. in the previous cross, representing a somewhat conspicuous difference as was expected.

On the Two-factor Hypothesis.

Hagiwara (1924, 1926) interpreted fasciation in terms of two factors, one of which was that for pear leaf. In my view, however, his data were not sufficient for establishing his hypothesis. The fact that his fasciated plants invariably bore pear leaves led him to conclude incorrectly that pear leaf was a manifestation of one of the fasciated factors, but this was owing to the fact that his data were not numerous enough to allow for breakage of the linkage. He postulated an ever-sporting nature of the factors concerned with the production of fasciation, but his conclusion, as far as I can judge, was surely taken from confused data.

The Lack of Physiological Fasciation.

The occurrence of non-heritable fasciation is a widespread phenomenon in various plant groups. In the Japanese morning glory, however, all fasciation would appear to have a factorial basis, and I have observed no single fasciated individual, which might have been physiologically produced during my thirteen years' culture of this plant. I have observed over three hundred thousand full-grown individuals, but I found no such fasciated plant! Hence we may conclude that the Japanese morning glory is a species in which physiological fasciation hardly, if ever, occurs.

THE BREAKAGE OF ACCOMPANIMENT OF FASCIATION AND PEAR LEAF.

Fasciation is almost always accompanied by pear leaf and this was also the case in old times. Fasciation seems to have made its appearance in pear leaves because specimens illustrated in the early literature invariably bore pear leaves (Fig. 9). Hagiwara (1924, 1926) regarded this accompaniment as a manifold effect of the pear leaf factor, which, combined with another factor, produces a fasciated stem. In my experiments, however, I obtained a few non-pear-leafed fasciateds in the hybrid progeny, so that we cannot retain this hypothesis any longer.

As was experimentally proved, my original fasciated pedigree strain, A 5, was pear leaf carrying the maple factor (Fig. 1, Pl. XVII, fig. 1). On crossing this strain with normals we produced some fasciateds in F_2, as indicated in Table XI; most of them bore pear leaves, but there were a few exceptions which bore non-pear leaves[1]. Such specimens were repeatedly observed in F_3 from the same cross (see Tables XII and XIII).

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[1] They produced no seeds.

How can we account for these exceptional individuals? We may, probably rightly, attribute them to the existence of crossing over between the factors for pear leaf and fasciation, in which case the next problem is to determine the fasciation factor in question and the linkage value.

Leaving the F_2 data for the moment, we may review the F_3 results of the cross 326 × A 5. Among the F_3 pedigrees we can recognise cases in which segregation on a dihybrid scheme occurred for pear leaf and its linked fasciation factor (the latter being one of the three fasciation factors). The families collected in Table XXI are regarded as recessively homozygous for the other fasciation factors, and so the appearance of fasciation is attributable to the segregation of one factor only.

TABLE XXI.

The F_3 data showing the dihybrid segregation of fasciation and pear leaf.

(From Tables XII and XIII.)

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total
49	43	1	—	12	56
60	21	—	—	5	26
79	78	4	—	24	106
83	77	2	1	17	97
86	36	1	1	14	52
101	17	—	—	4	21
129	70	2	—	26	98
133	98	3	1	21	123
146	17	—	1	3	21
176	86	—	2	26	114
178	27	—	—	8	35
Total	570	13	6	166	749
Expected	552·25	9·50	9·50	177·75	749

$$\chi^2 = 4·927. \quad P = 0·179.$$

In the total number, 749, I counted 166 fasciated specimens, or 22·16 per cent., which figure, as was stated above, must be somewhat augmented by taking into account the false normals. The deficit among the fasciateds on this account is, as pointed out earlier, about 11 per cent. If this value is taken in the present case, the 166 segregated fasciateds must be increased to *ca.* 184, *i.e.* 24·57 per cent. of the total, a simple recessive ratio. The occurrence of false normals may be justly expected in the present case, and the segregating number corrected accordingly. But the application does not seem to be so simple when we consider that the deficit may vary in different families according to the segregation

of modifiers. The fasciateds with non-pear leaves were not generally so evident in the flattening of their stems as those with pear leaves. One may therefore expect among them a somewhat higher deficit than among the pear-leafed fasciateds. The segregating numbers, if necessary, must be corrected under these conditions. So we have no alternative but to calculate the linkage value with the original data as they stand. From the total number in Table XXI, the gametic ratio is about 40 : 1, or 2·44 per cent. of crossing over. This strong linkage will lead to fasciated specimens being almost always accompanied by pear leaves in an experiment carried on a small scale. As indicated in the former section, we assumed three factors, f^1, f^2 and f^3, for fasciation, of which f^1 and f^2 are linked together with a medium frequency of crossing over. Now that we have discussed the occurrence of a close linkage between the two factors for pear leaf and for fasciation, a question arises as to which one of the three fasciation factors is linked with p. If either f^1 or f^2 is linked with p, these three factors must form a linkage group, while if f^3 is the one that is linked, then f^3 and p should segregate independently of f^1 and f^2. From an inspection of the F_3 data (see Tables XII and XIII) I have picked out families showing similar segregation and collected them in Table XXII.

The type of segregation is unusual in that the average frequency of the fasciated segregates is 13·91 per cent., and it can be identified with

TABLE XXII.

The F_3 data showing linkage in a complicated segregating type.

(From Tables XII and XIII.)

Pedigree number	Normal stem with non-pear leaf	Normal stem with pear leaf	Fasciated stem with non-pear leaf	Fasciated stem with pear leaf	Total
12	95	6	1	12	114
28	29	12	—	9	50
33	51	10	—	8	69
77	47	7	1	10	65
82	56	8	—	12	76
99	144	13	—	33	190
106	31	8	—	4	43
118	116	15	—	19	150
121	25	5	—	3	33
136	47	14	—	8	69
138	79	16	—	10	105
158	57	17	—	18	92
181	31	8	—	5	44
Total	808	139	2	151	1100
Expected	817·58	127·77	7·45	148·69	1101·49

$$\chi^2 = 5\cdot121. \quad P = 0\cdot165.$$

type V in Table XVII. In this type, the ratio of the normal and fasciated is 55 : 5, *i.e.* the proportion of the latter is about 14 per cent. The abnormal percentage of the fasciated is due to coupling between f^1 and f^2. If **p** segregated independently of these factors, it could not give such an unfamiliar ratio in these pedigrees. Consequently, we must conclude that f^1 (or f^2) is closely linked with **p**. Between f^1 and f^2 there is about 20–25 per cent. of crossing over, and, at the same time, between **p** and f^1 about 2·5 per cent. So these three factors may be considered to occupy loci on the same chromosome in a definite arrangement.

The Mode of Segregation of Maple and Variegation in Crosses involving Fasciation.

Hagiwara (1924) recognized two characters, maple and variegation, as linked with fasciation. The F_2 data, on which his consideration was based, did not seem to be enough to draw a conclusion, making it necessary to confirm his result with more data.

On the Maple Factor.

In Table XXIII, I have collected my data showing the segregation of maple and fasciation in fasciation crosses.

TABLE XXIII.

The F_2 data showing the segregation of fasciation and maple corolla.

Cross	Normal stem with perfect corolla	Normal stem with split corolla	Fasciated stem with perfect corolla	Fasciated stem with split corolla	Total
326 × A 5	485	135	14	5	639
81–1 × A 5	91	28	2	1	122
320 × A 5	46	10	1	0	57
350 × A 5	108	37	1	0	146
M 3 × A 5	168	58	1	0	227

The result seems not to point to any special relation between the characters in question. Hagiwara's F_2 data, however, showed a relatively high coupling. The flowers of pear leaves are sometimes irregularly deformed and broken, especially in the fasciateds (Pl. XVIII, fig. 8).

On the Variegation Factor.

The data showing segregation in variegation and fasciation are collected in Table XXIV from my fasciation crossings.

TABLE XXIV.

The F$_2$ data showing the segregation of fasciation and variegation.

Cross	Normal stem with self-coloured leaf	Normal stem with variegated leaf	Fasciated stem with self-coloured leaf	Fasciated stem with variegated leaf	Total
326 × A 5	481	139	8	11	639
320 × A 5	47	9	—	1	57
350 × A 5	113	32	—	1	146
M 3 × A 5	174	52	—	1	227

These crosses were made with a fasciated strain bearing variegated leaves as one of the parents, and therefore, a relatively high production of the parental type, if any linkage occurs, was expected. Actually a majority of the fasciated segregates bore variegated leaves, which indicated the occurrence of a special segregation. As formerly stated, fasciation was induced by the meeting of three recessive factors, f^1, f^2 and f^3, and it must be decided which of these three showed linkage, if any, with the variegation factor. If either f^1 or f^2 is linked with v, the variegation factor, this last factor should show linkage with p, the pear leaf factor, since f^1, f^2 and p are linked together. To determine this it

TABLE XXV.

The F$_2$ data showing the segregation of pear leaf and variegation in the crossing of **PV** × **pv**.

Cross	Self-coloured non-pear leaf	Variegated non-pear leaf	Self-coloured pear leaf	Variegated pear leaf	Total
M 3 × A 5	143	43	31	10	227
22–1 × A 5	44	17	12	3	76
350 × A 5	87	24	26	9	146
320 × A 5	44	9	3	1	57
326 × A 5	366	103	123	47	639
Total	684	196	195	70	1145
Expected	644·06	214·69	214·69	71·56	1145

$$\chi^2 = 5.943. \quad P = 0.115.$$

would be best to test a dihybrid segregation with pear and variegated leaves. The data obtained by **PV** × **pv** are collected in Table XXV, while Table XXVI gives the result of the cross **Pv** × **pV**. If linkage took place between the factors in question, we should expect coupling in the former table and repulsion in the latter, whereas independent segregation is actually the case in both tables. This test suggests that the fasciation factor which is linked with v is neither f^1 nor f^2, but possibly f^3. To verify this and the linkage value, I carefully inspected the F$_3$ data, but

TABLE XXVI.

The F_2 *data showing the segregation of pear leaf and variegation in the crossing of* **Pv** × **pV**.

Cross	Self-coloured non-pear leaf	Variegated non-pear leaf	Self-coloured pear leaf	Variegated pear leaf	Total
65 × BD–B	327	108	113	26	574
26–2 × BD–B	256	78	42	7	383
Total	583	186	155	33	957
Expected	538·31	179·44	179·44	59·81	957

$$\chi^2 = 19\cdot333. \quad P = 0\cdot0002*.$$

* This value is very low, mainly due to the excess and meagre productions in double dominant and double recessive classes.

I failed to secure any decided clue owing to the complicated segregation of the characters. Be that as it may, the F_3 data seem to show a linkage of the same order as the F_2 data, *i.e.* of about 20–25 per cent. of crossing over between f^3 and **v**. Hagiwara supposes the occurrence of a very high linkage (70 : 1 gametic ratio).

SUMMARY.

1. Pear leaf behaves as a simple recessive to the normal.

2. Pear leaf resembles cordate leaf in shape, but the genetic factors upon which these characters depend are entirely different.

3. The pear leaf factor combined with the "Rangiku" factor represents a particular leaf with a contracted and broadened petiole.

4. Pear leaf carrying the maple factor can be hardly distinguished from a normal pear leaf in appearance. The flower of the former, however, is split into lobes, while that of the latter is perfect, though sometimes it is deformed and slightly divided.

5. When homozygous for the "Rangiku" factor, the maple assumes the so-called "Mitsuo" leaf, the flower of which blooms in a split "Rangiku" way.

6. The pear leaf combined with the "Sasa" factor gives a slender, narrow leaf of the pear type, and a split corolla.

7. Though the original fasciated strain bred true to this abnormality, the fasciated specimens obtained in the hybrid progeny frequently give some normals.

8. A continuous variation in the degree of fasciation is exhibited among the segregates. In the most weakly fasciated specimens only a portion of the stem is flattened, and some genotypically fasciated plants, which remain normal throughout their growth, betray their nature by giving abundant fasciateds in their offspring.

9. Fasciation, which is a recessive character, occurs on a few individuals in F_2 from crosses with normals, the ratio of the fasciateds being only about 2 per cent. on an average.

10. In an examination of the experimental data we can detect two forms of segregating ratios with relatively higher and lower proportions of fasciated plants.

11. In the former case, the factors concerned with the production of fasciation are considered to be three, viz. f¹, f² and f³.

12. The factors f¹ and f² show linkage with about 20–25 per cent. of crossing over.

13. A marked variation in the degree of fasciation and in the appearance of false normals may be accounted for by the occurrence and segregation of a modifier or modifiers, which qualify the manifestation of the trait.

14. The lower proportion of fasciated plants in certain crosses is considered to be due to the occurrence of an additional fasciation factor, or of a modifier or modifiers.

15. The marked accompaniment of fasciated stem with pear leaf is due to linkage, with about 2·5 per cent. crossing over, between p (pear leaf factor) and f¹. The three factors, p, f¹ and f², therefore, may be considered to be located on the same chromosome in a definite arrangement.

16. The fasciation factor f³ is linked with v, a variegation factor, with about 20–25 per cent. of crossing over, thus constituting another linkage group.

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EXPLANATION OF PLATES XVII—XVIII.

Fig. 1. A variegated, pear-leafed specimen (A 5) bearing split flowers. Note the relatively broad split petals!

Fig. 2. A "Rangiku"-leafed specimen. Note the irregularly lobed leaves and disordered flower-buds!

Fig. 3. Variegated maple-"Sasa" leaf bearing a narrowly split flower divided down to the bottom of flower tube.

Fig. 4. "Rangiku" leaf bearing a creased flower. The gamopetalous corolla is unusually composed of numerous petals.

Fig. 5. "Mitsuo" leaf bearing a split flower, which, on account of the polypetalous constitution, is much divided.

Fig. 6. Maple leaf bearing split corollas. This type of flower is the same as that of Fig. 1.

Fig. 7. Pear-"Sasa" leaf with split flowers. Note the more or less closed funnel-shaped type of flower, and compare it with that of Fig. 9 on this plate. The somewhat closed corolla or "gentian" flower is an effect of the pear leaf factor.

Fig. 8. A pear-leafed specimen with a broadly fasciated stem. Note the disordered corollas!

Fig. 9. A pear-leafed and fasciated specimen carrying maple factors. Note the irregular and split corollas!

Fig. 10. Normal "Sasa" leaf bearing split flowers.

7

9

ON THE GENETICS AND CYTOLOGY OF FATUOID OR FALSE WILD OATS.

By C. LEONARD HUSKINS.

(1851 *Exhibition Science Research Scholar*.)

(With Three Plates and Fifty Text-figures.)

CONTENTS.

INTRODUCTION.

THE origin of the fatuoid or false wild forms which occur in cultivated oats, *Avena sativa* L., has been the subject of much study and controversy for many years. The literature of this subject prior to 1925 has been fairly extensively reviewed in a previous publication (Huskins and Fryer, 1925), but it may briefly be summarised here and other recent work included. According to Zade (1918), Haussknecht in 1884 was the first to describe these forms, which he termed "Zwischenformen" or *A. fatua* var. *transiens*, in accordance with his opinion that they were forms in process of transition from the wild oat *A. fatua* L., to the cultivated *A. sativa*. Koernicke and Werner (1885) described forms of oats similar to those of Haussknecht, and attributed their origin to natural crossing between *A. fatua* and *A. sativa*. Fischer (1900) studied similar forms from winter oats, but questioned whether they could have arisen from crossing. He considered it more probable that they were "throw-backs." Since that

time opinion has been sharply divided between the two opposing theories ascribing their origin to natural crossing or to some process of mutation.

Nilsson-Ehle (1911, 1921 *a*) has carried on extensive experiments with fatuoids from about 1900 to the present. He concludes that they originate by loss mutation. Howes (1908), Thellung (1911), Criddle (1912), Newman (1912, 1923), Åkerman (1921), Gante (1921), Marquand (1922), Garber (1922), Garber and Quisenberry (1923), Parker (1921) and Stanton, Coffman and Wiebe (1926) have all brought forward evidence favouring the theory of origin by mutation. Zade (1912, 1918) and Tschermak (1914, 1918) have been the chief exponents of the natural crossing theory. Crepin (1920), and Pridham (1924) have described forms intermediate between *A. fatua* and *A. sativa* which were almost certainly the products of natural crossing, and have discussed their relation to the fatuoid problem.

All recent work bearing directly on this problem, and much other work with an indirect bearing, indicates that the forms here described as fatuoids owe their origin to some sort of mutational process, but it is also clear that other forms intermediate between *A. fatua* and *A. sativa*, and readily confused with true fatuoids, do arise by natural crossing.

In general it seems safe to assume that all intermediate forms which on selfing give complex segregation involving a number of characters, have arisen from the crossing of *A. sativa* and *A. fatua*. Fatuoids, on the other hand, may be described as derivatives of *A. sativa*, or allied species that differ from the variety of cultivated oats in which they occur only in respect to the characters comprising the "fatuoid complex." In ordinary homozygous fatuoids this complex consists of a distinct articulation (or "sucker-mouth") surrounded by a tuft of hairs at the base of every grain, and a twisted, geniculate awn on the lemma of each grain. For such true-breeding forms the name *A. sativa* mut. *fatuoida*, has been suggested (Huskins and Fryer, 1925). Heterozygous fatuoids are intermediate in character between homozygous fatuoids and normal cultivated oats. They have the twisted, geniculate awn on only the primary grain of each spikelet, and the sucker-mouth and basal hairiness are much reduced. Homozygous fatuoid, heterozygous fatuoid, and normal segregates from four different strains of heterozygous fatuoids are shown in Plates XIX and XX.

In nearly all recorded cases the heterozygous form appears first. Marquand (1922), however, has described the direct appearance of the homozygous form from one seed from a normal plant of the Victory variety. All the sister seeds produced normal plants.

Heterozygous fatuoids usually segregate homozygous fatuoids, heterozygous fatuoids, and normal oats, in a ratio of approximately 1 : 2 : 1. Goulden (1926), however, found two other ratio types, and divergent types have been discovered in the course of the present study. One of Goulden's divergent types gave dwarf sterile homozygous fatuoids, heterozygous fatuoids, and normals in the ratio of 5 : 15 : 20. The other gave the same classes of segregates in the ratio of 60 : 63 : 15.

The term "homozygous fatuoid" has become generally applied to the form showing the fully developed fatuoid characters, since these forms have until recently always been found to breed true. Stanton, Coffman and Wiebe (1926), have, however, described fully developed fatuoids from Fulghum, a variety now classed as *A. byzantina*, which do not breed true. It seems possible that this may be the result of natural crossing, which has been shown to be especially common in this variety, though, as will be mentioned later, it might also result from chromosome aberration. At any rate, until the possibility of natural crossing has been eliminated, it does not seem necessary to adopt a new terminology such as Stanton *et al.* propose. In this paper the fully developed fatuoid forms will therefore, in conformity with general usage, be referred to as homozygous fatuoids, or, more briefly, as hom. fatuoids, and the intermediate forms as heterozygous or het. fatuoids.

In the course of the present study it has become obvious that the analogy between the fatuoid forms of oats and the speltoid forms of wheat is a very close one. Results of investigations on speltoids, of which there have been many extensive studies, will therefore be discussed, and an attempt will be made in a general way to correlate with the speltoid and fatuoid data some of the extensive genetical and cytological results available from hybridisation studies in wheat and oats.

Material and Methods.

The fatuoid strains used have been obtained from many sources. The origin of each is given with the other data concerning it.

Belling's (1921) iron-aceto-carmine method, and a modification of it, was used for some early cytological observations and for a few recent comparative studies. In the modified method, material is fixed in Carnoy's fluid, and kept in 75 per cent. alcohol until required; slides are then prepared as in Belling's second method for fresh material, and sealed with a solution of gum-damar in xylol.

The cytological figures in this paper are mainly from permanent paraffin sections. Two are from permanent smears prepared by a method

similar to that of Taylor (1924) except that Flemming fixative and iodine-gentian-violet stain are used, and three of pollen "tetrads" are from aceto-carmine preparations.

Bouin's fluid as modified by Allen (75 c.c. saturated aqueous picric acid solution, 25 c.c. commercial formalin, 5 c.c. glacial acetic acid, 2 grm. urea crystals, 1·5 grm. chromic acid) was the fixative used for practically all of the preparations figured. Carnoy, Flemming, ordinary Bouin, and Navashin fixatives have been used to a limited extent.

Heidenhain's iron-alum-haematoxylin and iodine-gentian-violet have been used about equally for staining, and Cole's (1926) rapid haemotoxylin to a small extent. The iodine-gentian-violet method is particularly valuable for the interpretation of overlapping chromosomes. Mr W. C. F. Newton of the John Innes Horticultural Institution devised this adaptation of the Gram method some four years ago, but though it is now used by a number of workers, particulars of the original method have not previously been published. Preparations are stained in a 1 per cent. boiled and filtered aqueous gentian-violet solution for from 4 to 7 minutes. They are then rinsed rapidly in water and placed in a 1 per cent. iodine and 1 per cent. potassium iodide in 80 per cent. alcohol solution for about 30 seconds. From this they are passed almost as rapidly as possible through two jars of absolute alcohol, into clove oil, differentiated under the microscope, and passed into xylol. The best results are obtained after chrom-osmic fixatives or Allen's Bouin. For most other fixatives, modifications such as those of Clausen (1926) have to be adopted.

Sections have been cut at 10μ–24μ. Owing to the large number of chromosomes, their bulk, and variation in size and form, it is usually difficult, even with the iodine-gentian-violet stain, to get clear interpretations of irregular chromosome structures in whole cells at diakinesis, at which stage the nuclear diameter is 20μ or more. Cut cells have, therefore, to be used to a considerable extent in the study of this stage. A Zeiss 2 mm. apo. objective, 20 × ocular, and camera lucida were used for all drawings showing chromosomes. At the drawing-table height used, this gave a magnification of 2550.

Examinations of pollen have been made on ripe anthers fixed in Carnoy's, and preserved in 75 per cent. alcohol. Single anthers were then placed in a small drop of warm 2 per cent. gelatine on a slide, cut across, the pollen pressed out, and a cover glass large enough to cover all the fluid, applied. In doubtful cases all the grains on a slide were counted. Distinction has been made only between dark grains, and empty, trans-

parent ones. In some cases there are degrees of density in the dark grains which may indicate differences in viability, but pollen germination tests have not been made. Numerous check counts were made on some of the strains which gave very variable results, but only three or four of the largest and most representative counts are shown in the table.

The calculations on relative vigour of the different segregates from het. fatuoids are based on the number of culms bearing mature heads, and upon the height of the tallest culm of each plant. Since only the immediate progeny of het. fatuoids are included, the different segregates measured were growing intermingled and should all be affected proportionately by all external influences affecting vigour. All plants were grown in rows 6 inches apart, and spaced 4 inches apart in the rows. The border plants were very little more vigorous than the inside ones, but where the number is large enough to permit it, as in strains 24–20, 25–29, and 25–46, the border plants have been omitted from the calculations. Inclusion of them would increase all the means and standard deviations very slightly, but should leave the relative differences unchanged, since all classes of segregates are affected proportionately. The calculations upon het. fatuoid Kanota 26–10, 11, 12, and 16, have been made both with and without border plants to show their effect.

Winter generations have been grown in some cases to hasten genetic results. In Tables I, II and III all autumn sowings are greenhouse sowings. For the cytological studies field-grown material has been used almost exclusively.

Each plant propagated is given an individual number, but when related plants are discussed collectively they are designated by the strain number of the original plant from which they are descended.

Normal Oat Species.

A number of different species and varieties of oats have been studied cytologically for comparison with fatuoids, and the following chromosome numbers, some of which have previously been published (Huskins, 1925, 1926) have been determined from counts in pollen-mother-cells. *Avena brevis*[1], *A. strigosa*[1], *A. Wiestii*[1] have 7/14 chromosomes; *A. barbata*[1] (Cornell strain) 14/28; *A. sativa*[1] vars. Banner, Victory and Lincoln, *A. sativa orientalis*, *A. sativa gigantica* (Cornell), *A. nuda*[1], *A. byzantina*[1, 2], *A. fatua*[1, 2], *A. sterilis*[1, 2] and *A. ludoviciana*[2] have 21/42.

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[1] The counts for these species agree with those of one or more of the following authors: Kihara (1919, 1924), Nikolaewa (1922), Winge (1925), Dorsey (1925) and Stolze (1925).

[2] Nikolaewa (1922) from somatic counts has given higher numbers for these species.

These species thus fall into three distinct groups, a situation similar to that in the genus *Triticum*, except that in it many more species with the intermediate number, 14/28 are known. In both genera there is considerable evidence that the species with the higher number of chromosomes are derived from those with the lower. In all the phylogenetically hexaploid species and varieties of oats examined, however, the cytological behaviour is similar to that of normal diploid species, the 42 chromosomes regularly forming 21 bivalents at diakinesis. In *A. fatua* especially, the divisions proceed with almost diagrammatic regularity, and the pollen formed is practically all "good." The percentage of empty pollen grains found is negligible, as shown in the three counts given in Table V. In none of the three varieties of *A. sativa* examined are the reduction-divisions quite as regular as in *A. fatua*, but no irregularities of apparent significance have yet been found. The twenty-one bivalent chromosomes nearly always split practically simultaneously in the first anaphase, but occasionally laggard and vagabond chromosomes are seen. The second division follows closely upon the first and is nearly always regular. The resultant microspore tetrads are of the bilateral type, and no deviation from this arrangement of the microspores has yet been found in the pure species. The pollen does not seem to be as uniformly good as in *A. fatua*, but the percentage of empty grains is still quite small. In the three counts of Banner pollen shown in Table V, it ranges from 3·24 to 3·78 per cent.

FATUOIDS OF TYPE 1.

Under Type 1 are grouped all the heterozygous fatuoids, whether of direct origin or derived from crosses between hom. fatuoids and normal oats, which segregate hom. fatuoids, het. fatuoids and normals, of approximately equal vigour and in a ratio of approximately 1 : 2 : 1. This is by far the most common type of fatuoids and the only one that has been described prior to the present work and that of Goulden (1926), though different types of the closely analogous speltoids have been known for many years. Plate XIX, strain 26–24, and Plate XX, strain 26–10, are typical examples, showing the grain characters and relative size of panicle of the three classes of segregates from Type 1 het. fatuoids. The other features shown such as colour, panicle shape, and absolute size of panicle are, of course, varietal characters having no relation to the fatuoid question.

Strain 24–20, on which the most data are available, originated from a single heterozygous fatuoid panicle found in a head-selection plot of

TABLE I.

Record of Heterozygous fatuoid 24–20 and Progeny.

Parent plant	Uncontrolled or selfed	Strain number	Date sown	Number of seeds	Number germinated	Homozygous fatuoid	Heterozygous fatuoid	Normal	Unclassified	Total
Het. fatuoid from head-selection plot, Banner oats, Univ. of Alberta	U.	24–20	15. ix. 21	14	—	1	3	2	—	6
	U.	,,	24. ii. 25	16	—	2	6	7	—	15
	U.	,,	19. v. 25	30	—	7	3	5	—	15
	U.	,,	22. ii. 26	11	6	0	5	1	—	6
Heterozygous fatuoid from 24–20	U.	25–33	5. vi. 25	32	—	6	16	9	—	31
,, ,, ,,	U.	,,	22. ii. 26	22	21	7	10	3	—	20
,, ,, ,,	U.	25–34	5. vi. 25	32	—	5	17	3	—	25
,, ,, ,,	U.	,,	22. ii. 26	11	11	4	4	2	—	10
,, ,, ,,	U.	25–35	26. v. 25	64	—	4	5	4	—	13
,, ,, ,,	U.	*25–35 a	5. vi. 25	128	—	19	61	28	—	108
,, ,, ,,	U.	,,	22. ii. 26	110	109	29	45	28	—	102
,, ,, ,,	U.	*25–35 b	5. vi. 25	128	—	30	54	32	—	116
,, ,, ,,	U.	,,	22. ii. 26	88	86	19	42	25	—	86
,, ,, ,,	S.	25–101	,,	99	94	18	37	35	—	90
,, ,, 25–33	U.	25–146	24. ii. 26	154	117	19	48	25	—	92
,, ,, 25–34	U.	25–138	,,	264	175	27	70	47	—	144
,, ,, 25–35	U.	25–128	,,	143	80	18	26	14	—	58
,, ,, ,,	U.	25–134	,,	77	69	16	25	15	—	56
,, ,, ,,	U.	†25–154	11. iii. 26	77	76	17	35	19	5	76
,, ,, ,,	U.	†25–157	24. ii. 26	33	31	7	16	7	—	30
,, ,, ,,	U.	†25–155	,,	22	22	4	10	7	1	22
,, ,, ,,	U.	,,	8. iii. 26	22	20	8	6	3	—	17
$P (1:2:1) = 0{\cdot}0005$ Total:				1577	917	267	544	321	6	1138
$P (1:3) = 0{\cdot}2721$ Expected $(1:2:1) =$					$+x$	283	566	283	—	1132
Homozygous fatuoid from 24–20	U.	25–32	5. vi. 25	32	—	All	—	—	—	x
,, ,, ,,	U.	,,	22. ii. 26	11	3	2	—	—	—	2
,, ,, ,,	S.	25–100	,,	33	32	29	—	—	—	29
,, ,, ,,	S.	,,	21. iii. 26	44	39	24	—	—	—	24
,, ,, 25–33	U.	25–147	22. ii. 26	22	11	8	—	—,	—	8
,, ,, 25–34	U.	25–140	,,	22	17	16	—	—	—	16
,, ,, 25–35 b	U.	25–127	,,	11	6	6	—	—	—	6
,, ,, ,,	U.	25–132	,,	22	18	17	—	—	—	17
,, ,, ,,	U.	25–135	,,	22	18	17	—	—	—	17
,, ,, 25–32	U.	25–131	,,	11	11	10	—	—	—	10
,, ,, ,,	U.	25–142	,,	11	4	4	—	—	—	4
,, ,, 25–36	U.	25–143	,,	11	7	5	—	—	—	5
Total:				210 $+32$	166 $+x$	138 $+x$	—	—	—	138 $+x$
Normal segregate from 24–20	S.	25–99	24. ii. 26	22	22	—	—	22	—	22
,, ,,	S.	,,	29. iii. 26	121	120	—	—	94	—	94
,, ,, 25–33	U.	25–145	24. ii. 26	22	19	—	—	19	—	19
,, ,, 25–34	U.	25–141	,,	22	21	—	—	18	—	18
,, ,, 25–35 a	U.	25–129	,,	22	14	—	1	12	—	13
,, ,, ,,	U.	25–133	,,	22	19	—	—	19	—	19
,, ,, ,,	U.	25–136	,,	22	22	—	—	22	—	22
Total:				253	237	—	1	206	—	207

* 25–35 a and 25–35 b are primary, awned, and secondary, awnless grains respectively, from the same plant.

† 25–154 and 25–157 were classed at harvest time as "probable het.," and 25–155 as "possible normal."

Banner Oats in the Department of Field Husbandry Investigation Field, University of Alberta, Edmonton, Canada. The genetical data from this strain are assembled in Table I. The deviation from the expected 1 : 2 : 1 ratio is seen to be great. The χ^2 test for goodness of fit (*Tables for Statisticians*, Pearson, 1911) gives $P = \cdot0005$, *i.e.* only 5 times in 10,000 would such deviation be expected to occur solely by chance.

Such deficiences in the fatuoid classes of segregates have been reported by nearly all workers with fatuoids, and they have generally assumed that it occurs through the mutant plants being less vigorous than the normals, and more easily destroyed by any unfavourable environmental conditions. The present evidence does not support this assumption. The statistical study of relative vigour shows in general that there is little if any significant difference in vigour (as measured by height and number of culms per plant) between the different classes of segregates from Type 1 het. fatuoids, though in fatuoids of the other types there are decided differences. The data on this point are assembled in Table IV, and are discussed in detail in the account of each strain.

Direct evidence that the deficiency in the fatuoid classes is not at any rate due solely to selective elimination by environmental conditions is obtained by picking out from Tables I, II and III, 15 sowings which have given the highest germination and survival rates. From the total of 951 seeds in these 15 sowings, 921 seeds germinated, and 895 mature plants were obtained. These 895 plants comprise hom. fatuoids, het. fatuoids and normals in the ratio of 207 : 432 : 256. In relation to the normal class there is here (neglecting probable error) a deficiency from the expected 1 : 2 : 1 ratio, of 80 het. fatuoids, and 49 hom. fatuoids, while only 56 fewer mature plants were produced than there were seeds sown.

It will be noted that a number of sowings gave very low germination rates. Such sowings are mainly seeds from late-sown plants which were damaged by frost before reaching maturity. Germination counts were not made prior to 1926. In 1926 counts were made at various times up to about six weeks after sowing. The highest count found during this period is given in the tables as the "number germinated," though a few seeds may have germinated after the last count. The low survival rate in 1926 of some strains sown later than about the end of March is due to attack by the frit fly, *Oscinis frit*.

Selective elimination of fatuoid male gametes during sporogenesis or fertilisation is believed to be the major cause of the deficiency in the fatuoid classes. Pollen counts (Table V) show that there is a much

larger percentage of empty pollen in the segregates from het. fatuoids than in normal *A. sativa* or *A. fatua*, and though the results are very variable, the percentage is usually greater in hom. and het. fatuoid segregates than in normal sibs.

If fatuoid gametes for any reason do function less frequently than normal gametes, this would account for the deviation from 1 : 2 : 1, and for the fact that when the het. fatuoid and normal classes are added together and considered in relation to the hom. fatuoid class, the progeny of this het. fatuoid Banner strain 24–20 produce a fair 3 : 1 ratio [865 : 267, Expected = 849 : 283, $P = \cdot27$ (Yule, *Fourfold Tables*, 1926)].

While hom. fatuoids can always be classified without difficulty, it is not always easy to distinguish between het. fatuoid and normal plants. In some strains very little difficulty occurs, but in others a progeny test has to be made before some of the plants can be classified with certainty. Environmental conditions very greatly affect the development of the awns in genetically normal segregates or pure varieties of *A. sativa*. If a number of genetically het. fatuoid plants happened to be classed as normal this would, of course, account for the deviation from the 1 : 2 : 1, and the conformity to the 3 : 1 ratio. It happens that in the strain No. 24–20 distinction of the het. fatuoid and normal segregates usually seems to be quite easy. In the 1925 work only three plants that seemed doubtful were found. Two of these, 25–154 and 25–157 were classed as "probable het." and the 1926 progeny test proved them to be het. fatuoid. One plant, 25–155, was classed as "possible normal," and turned out to be het. fatuoid. As shown in Table I, 14 other plants definitely classed at harvest as het. fatuoid or normal have proved in all cases to have been correctly classified.

The primary awned grains and secondary awnless grains from one plant, No. 25–35, of this strain were sown separately. As shown in Table I, their progeny ratios are similar.

The 9 hom. fatuoid segregates from this strain which have been propagated have all bred true. The total number of progeny of one of them, 25–32, was apparently not recorded in 1925, the only entry that can now be found being "all hom. fatuoid." The total progeny of these 9 segregates can therefore be given only as 138 + .

The 6 normal segregates from this strain, recorded in Table I, have produced a total of 206 normal progeny and 1 het. fatuoid plant. Since the parent of this het. fatuoid was not artificially selfed it may have arisen as the result of natural crossing.

The calculations on relative vigour of the different segregates of

this strain (Table IV), show similar results in 1925 and 1926. In neither year is there any significant difference in the height of the different classes, but in both years the standard deviation of the height is greatest in the hom. fatuoid class. The difference, though small, is probably significant in the 1925 results, and possibly so in 1926. The coefficient of variation of height gives the same results. The number of culms is smallest in the hom. fatuoids in both years, and the difference is possibly significant in each case. There is no significant difference in the standard deviation of the number of culms in either year.

Since the cytological findings are similar in all strains of fatuoids of Type 1 which have been examined, they will be described together at the end of this section after the genetic data from other strains have been presented.

Het. fatuoid Newmarket, strain 25–82, originated from a het. fatuoid plant found in 1925 at the University of Alberta in a sowing of hom. fatuoid seeds from the variety Newmarket. These seeds were obtained from Mr Norman Criddle, Dominion Department of Entomology, Ottawa, who had collected them about 1910 when he was studying fatuoids. Table II shows the progeny record of different panicles of this het. fatuoid plant. No. 25–82 a is seed from a single panicle which was covered with a manila bag during its flowering period. No. 25–82 b is seed from the remaining panicles of this plant in which pollination was not artificially controlled. This difference in treatment should not, of course, affect the progeny ratios, as oats under most conditions are very closely self-fertilised. There is, however, a striking difference in the ratios from the two sowings. Together they form a very good 1 : 2 : 1 ratio, $\chi^2 = 0.189$ (P = close fit), but separately the deviation is great. For 25–82 a $P = 0.025$. Though the numbers are small the fact is of interest, especially when considered in relation to Garber's (1922) results with Victory and Garton 784 fatuoids. His het. fatuoid Victory strain gave hom. fatuoids, het. fatuoids, and normals in the ratio 80 : 163 : 54 ($P = 0.0256$), while het. fatuoid Garton gave 55 : 122 : 81 ($P = 0.0498$). But considered together, the ratio is 135 : 285 : 135 ($\chi^2 = 0.4053$; P = close fit).

The calculations upon height and number of culms, Table IV, show that there are no significant differences in these respects between the different segregates from this het. fatuoid Newmarket strain, 25–82.

Het. fatuoid Banner type, strain 24–26 (Table II) originated from a het. fatuoid plant found growing on waste land on the University Farm, Edmonton. The three classes of segregates from this strain are of

apparently equal vigour, and their numbers, 25 : 56 : 34, constitute a good 1 : 2 : 1 ratio ($\chi^2 = 1\cdot42$; $P = \cdot51$). Both the artificially selfed and the uncontrolled panicles of the het. fatuoid plant 25–93, selected from this strain in 1925, gave practically perfect germination and survival rates in 1926. There is no possibility of appreciable selective elimination of any type of segregate here, and no significant difference in the ratios from the artificially selfed and the uncontrolled seeds.

TABLE II.

Record of various Heterozygous fatuoid Strains of Type 1.

				Progeny						
Parent Plant	Uncontrolled or selfed	Strain number	Date sown	Number of seeds	Number germinated	Homozygous fatuoid	Heterozygous fatuoid	Normal	Unclassified	Total
Het. fatuoid Newmarket; seed from N. Criddle	S.	25–82 a	22. ii. 26	99	95	31	46	13	—	90
Different panicles of same plant	U.	25–82 b	11. iii. 26	77	77	12	37	26	—	75
Total:				176	172	43	83	39	—	165
$P=$close fit		Expected (1 : 2 : 1) =				41·25	82·5	41·25	—	165
Het. fatuoid, Banner type, from waste land, Edmonton	U.	24–26	24. ii. 25	2	2	1	1	—	—	2
"	U.	"	19. v. 25	10	—	—	6	2	—	8
Het. fatuoid from 24–26	S.	25–93 a	22. ii. 26	66	66	18	29	18	—	65
" " "	U.	25–93 b	11. iii. 26	44	44	9	20	14	1	44
Total:				122	112	28	56	34	1	119
$P=0\cdot51$		Expected (1 : 2 : 1) =			$+x$	29·5	59	29·5	—	118
Het. fatuoid Victory; seed from R. J. Garber	U.	25–52	22. ii. 26	22	8	3·	4	1	—	8
Het. fatuoid Garton 784; seed from R. J. Garber	U.	25–55	22. ii. 26	33	15	6	2	7	—	15
Het. fatuoid Aurora; seed from R. J. Garber	U.	25–58	22. ii. 26	33	28	4	16	8	—	28
" " "	U.	25–59	"	22	21	7	8	5	—	20
" " "	U.	25–60	"	11	10	1	5	4	—	10
Total:				66	59	12	29	17	—	58
Het. fatuoid Kanota; seed from J. H. Parker	U.	26–10	24. ii. 26	99	92	21	46	25	—	92
" " "	U.	26–11	"	55	40	12	17	11	—	40
" " "	U.	26–12	"	55	53	11	29	12	—	52
" " "	U.	26–16	"	22	19	4	8	6	—	18
Total:				231	204	48	100	54	—	202
$P=$close fit		Expected (1 : 2 : 1) =				50·5	101	50·5	—	202

Het. fatuoid Banner strain 24–21 originated similarly to 24–20 from a het. fatuoid panicle found in a head-selection plot of Banner oats at Edmonton. Unfortunately the few seeds on this panicle germinated badly and gave only two hom. fatuoids, one clearly normal plant and two doubtfuls which were classed as "probable het." Both these doubtful

plants, 25–37 and 25–91, turned out to be genetically normal. Five hundred seeds from them and the clearly normal plant all produced only normal type offspring. One hundred and sixty-four seeds from the two original hom. fatuoids, and two of their hom. fatuoid progeny produced all hom. fatuoid plants except one which was het. fatuoid. As in the case of the het. fatuoid plant which arose from seed of a normal segregate of strain 24–20, the parent plant was not artificially selfed. The normal segregates of this strain are all much more heavily awned than ordinary Banner oats grown under similar conditions. Since no segregation ratios are available, this strain can be classified only on the basis of the comparative vigour of its hom. fatuoid and normal segregates, which does not differ, and on its cytology. The latter is of interest in view of the fact that normal plants were studied under the mistaken impression that they were het. fatuoids.

Het. fatuoid Victory, strain 25–52, het. fatuoid Garton 784, and het. fatuoid Aurora, Table II, are all from seed obtained from Professor R. J. Garber, West Virginia Agricultural Experiment Station, Morgantown, Va. They have been grown only on a small scale for cytological purposes, as their genetic behaviour has already been studied (Garber, 1922).

Garber and Quisenberry (1923) have also shown that delayed germination, which characterises *A. fatua*, is absent from these strains of fatuoids. The Victory and Garton 784 het. fatuoid strains germinated very badly in 1926 and were severely attacked by disease, bacterial blade blight probably being the principal affection. The Aurora het. fatuoid strains germinated well and were disease resistant.

Seeds of a number of strains of fatuoids from the variety Kanota were obtained from Professor John H. Parker, Kansas Agricultural Station, Manhattan, Kansas. Kanota is a selection from the variety Fulghum, and with it is generally considered to belong to the species *A. byzantina* C. Koch. Four of the het. fatuoid strains obtained, Nos. 26–10, 26–11, 26–12, and 26–16, appear to be similar to Type 1 het. fatuoids from *A. sativa*, except, of course, for their particular varietal characteristics which include extreme earliness, shortness of straw, small size of panicle, and yellowish colour of the grain. Typical panicles of the three classes of segregates from 26–10 are shown on Plate XX. As shown in Table II, these strains germinated well, and gave good 1 : 2 : 1 ratios. Together they gave 48 : 100 : 54 (P = close fit). Table IV shows the het. fatuoid segregates to be slightly taller than the normals, both when the border plants of the plots are included and when they are excluded, but the differences are not statistically significant. The hom. fatuoids in both

cases are shorter than either of the other classes, and the difference is possibly significant in each case. The standard deviation of the height is least in the het. fatuoids, the differences again possibly being significant. It is greatest in the normals, this result being due to the occurrence of one normal plant only 26 inches high. The coefficient of variation gives similar results. No significant differences appear in the number of culms. The standard deviation of the number of culms seems to be significantly higher in the hom. fatuoid class in the calculations both with and without the border plants. The cytology of these strains of fatuoids from *A. byzantina* seems to be identical with that of Type 1 fatuoids from *A. sativa* with respect to the features characteristic of fatuoids, and will be described with that of the others.

Reciprocal crosses between a strain of pure Banner oats, and one of hom. fatuoids of the Banner type were made in 1924. The F_1 plants were grown in the greenhouse at Edmonton during the winter 1924–25. They appeared to be in every way similar to ordinary het. fatuoids of the Banner type. The seeds of the F_1 plant Hom. fatuoid × Banner were given the strain number 25–29, and the reciprocal was numbered 25–46. The F_2 generation was grown in the field in 1925 and 1926, and seeds from some of the 1925 F_2 segregates were also sown in 1926. The results are shown in Table III. As with strain 24–20, there is considerable deviation from the expected 1 : 2 : 1 ratio ($P = \cdot 0082$). But again, when the het. fatuoids and normals are classed together and considered in relation to the hom. fatuoids, a good 3 : 1 ratio is obtained (602 : 187; $P = \cdot 97$). In these strains, 25–29 and 25–46, classification of the different segregates seems to be even easier than in strain 24–20. No plants were classified as doubtful in 1925, and all plants propagated in 1926 proved to be correctly classified. The pollen counts (Table V), though again very variable, show clearly that a larger percentage of empty pollen is formed by segregates of het. fatuoids than by pure varieties of *A. sativa* or *A. fatua*, and both the het. and hom. fatuoid segregates have worse pollen than the normal sibs. Selective elimination of fatuoid gametes is therefore again indicated as the cause of the deviation from 1 : 2 : 1. With almost perfect germination and survival rate in several sowings of each of the three classes of segregates, as shown in Table III, there apparently cannot be any appreciable selective elimination of fatuoids during either the germination or the growth period. The calculations on relative vigour of the different classes of segregates (Table IV) show no differences that are definitely significant either in 1925 or 1926, though several differences verge on significance. In 1925 the standard deviation and coefficient of

TABLE III.

Records of Hybrids of Hom. fatuoids × Normal Oat Varieties, and Progeny.

Parent plant	Uncontrolled or selfed	Strain number	Date sown	Number of seeds	Number germinated	Homozygous fatuoid	Heterozygous fatuoid	Normal	Unclassified	Total
F_1 Hom. fatuoid × normal Banner grown in greenhouse 1924–25 24–32	U.	25–29	26. v. 25	128	—	22	43	26	—	91
	U.	25–113	22. ii. 26	110	103	29	41	26	—	96
Het. fatuoid F_2 segregate of 25–29	U.	25–113	24. ii. 26	110	103	21	38	33	—	92
,, ,, ,,	U.	25–118	,,	110	97	20	45	23	—	88
,, ,, ,,	U.	25–125	,,	44	40	9	18	8	—	35
F_1 Normal Banner × Hom. fatuoid grown in greenhouse 1924–25 24–33	U.	25–46	26. v. 25	32	—	7	8	7	—	22
	U.	25–109	22. ii. 26	110	106	18	56	30	—	104
Het. fatuoid F_2 segregate of 25–46	U.	25–109	,,	99	85	26	37	16	—	79
,, ,, ,,	U.	25–119	24. ii. 26	121	117	19	44	35	—	98
,, ,, ,,	U.	25–123	,,	99	90	16	37	31	—	84

$P (1 : 2 : 1) = ·0082$ Total:
$P (1 : 3) = ·0728$ Expected $(1 : 2 : 1) =$

				Number of seeds	Number germinated	Homozygous fatuoid	Heterozygous fatuoid	Normal	Unclassified	Total
Total				963	741	187	367	235	—	789
Expected (1 : 2 : 1)					$+x$	197·25	394·5	197·25	—	789

Parent plant	Uncontrolled or selfed	Strain number	Date sown	Number of seeds	Number germinated	Homozygous fatuoid	Heterozygous fatuoid	Normal	Unclassified	Total
Hom. fatuoid F_2 segregate of 25–29	U.	25–110	22. ii. 26	44	41	40	—	—	—	40
,, ,, ,,	U.	25–120	,,	22	22	22	—	—	—	22
,, ,, ,,	U.	25–126	,,	22	21	21	—	—	—	21
,, ,, 25–46	U.	25–111	,,	44	40	39	—	—	—	39
,, ,, ,,	U.	25–115	,,	22	22	22	—	—	—	22
,, ,, ,,	U.	25–122	,,	22	22	21	—	—	—	21
Total:				176	168	165	—	—	—	165
Normal F_2 segregate of 25–29	U.	25–114	24. ii. 26	22	21	—	—	19	—	19
,, ,, ,,	U.	25–116	,,	22	22	—	—	22	—	22
,, ,, ,,	U.	25–124	,,	22	18	—	—	18	—	18
,, ,, 25–46	U.	25–112	,,	22	21	—	—	21	—	21
,, ,, ,,	U.	25–117	,,	22	6	—	—	6	—	6
,, ,, ,,	U.	25–121	,,	22	18	—	—	14	—	14
Total:				132	106	—	—	100	—	100
F_1 Hom. fatuoid × normal Sir Douglas Haig. Cross made by R. J. Chittenden and F_1 grown 1923	U.	26–24	1. iii. 26	154	137 Expected = 30 $P (1 : 2 : 1) = ·45$	36 30	55 60	29 30	—	120
F_1 Hom. fatuoid × Banner. Cross made and F_1 grown by C. H. Goulden	U.	26–43	16. iv. 26	88	76 Expected = 8 P =good fit.	9 8	15 16	8 8	—	32

variation of height of the hom. fatuoid segregates were greater than those of the other segregates, but the situation was reversed in 1926.

Seed of a similar F_1 hybrid, Hom. fatuoid × Banner, was obtained from Dr C. H. Goulden, Dominion Rust Research Laboratory, Winnipeg, who made the cross and grew the F_1 plant at the University of Saskatchewan several years ago. Unfortunately these seeds were received and sown too late in 1926 to give satisfactory results. They were very badly

TABLE IV.

*Calculations on the relative vigour of the different classes of segregates
from various strains and types of het. fatuoids.*

Strain and Year	Segregates	No.	Mean height	S.D. of height	C. of V. of height	Mean No. of culms	S.D. of No. of culms
Het. fatuoid Banner, No. 24-20, 1925 crop	Hom. fatuoid	64	49·26± ·37	4·42±·26	8·98± ·53	3·97±·16	1·93±·12
	Het. fatuoid	153	49·29± ·16	2·99±·12	6·07± ·24	4·50±·11	2·01±·08
	Normal Banner	76	49·70± ·19	2·42±·13	4·97± ·12	4·46±·16	2·01±·11
Het. fatuoid Banner, No. 24-20, 1926 crop	Hom. fatuoid	150	55·39± ·41	7·51±·29	13·56± ·54	2·07±·05	0·92±·04
	Het. fatuoid	288	55·14± ·25	6·23±·18	11·29± ·32	2·32±·04	1·04±·03
	Normal Banner	183	54·81± ·34	6·82±·24	12·44± ·45	2·42±·06	1·13±·04
Het. fatuoid ex Hom. fatuoid × Banner. No. 25-29, 1925 crop	Hom. fatuoid	22	41·23± ·72	4·99±·51	12·10±1·25	4·32±·29	1·99±·20
	Het. fatuoid	43	43·19± ·33	3·24±·24	7·50± ·55	4·81±·18	1·76±·13
	Normal Banner	26	42·81± ·53	4·01±·38	9·37± ·88	5·89±·42	3·18±·30
Het. fatuoid ex Hom. fatuoid × Banner, No. 25-29, and the reciprocal, No. 25-46, 1926 crop	Hom. fatuoid	135	58·50± ·25	4·28±·18	7·32± ·30	2·44±·06	1·08±·04
	Het. fatuoid	243	58·81± ·20	4·61±·14	7·84± ·24	2·44±·04	1·00±·03
	Normal Banner	159	59·08± ·28	5·33±·20	9·02± ·34	2·30±·05	0·93±·04
Het. fatuoid Newmarket, No. 25-82, 1926 crop	Hom. fatuoid	43	54·68± ·49	4·74±·34	8·67± ·64	2·44±·08	0·78±·06
	Het. fatuoid	83	55·78± ·37	5·08±·26	9·11± ·48	2·44±·07	0·92±·05
	Normal Newmarket	39	55·74± ·57	5·30±·40	9·51± ·73	2·20±·08	0·79±·06
Het. fatuoid Kanota, Nos. 26-10, -11, -12, and -16, 1926 crop. Border plants included	Hom. fatuoid	48	40·33± ·33	3·42±·24	8·48± ·59	4·21±·18	1·87±·13
	Het. fatuoid	100	42·31± ·18	2·63±·12	6·22± ·30	4·21±·10	1·56±·07
	Normal Kanota	54	42·18± ·39	4·21±·27	9·98± ·65	4·04±·13	1·38±·09
Het. fatuoid Kanota, Nos. 26-10, -11, -12, and -16, 1926 crop. Border plants excluded	Hom. fatuoid	30	39·87± ·39	3·16±·28	7·92± ·69	4·00±·20	1·63±·14
	Het. fatuoid	87	42·34± ·18	2·47±·13	5·83± ·30	3·95±·09	1·19±·06
	Normal Kanota	46	41·65± ·42	4·25±·30	10·20± ·72	3·85±·13	1·34±·09
Het. fatuoid Kanota, No. 26-13, 1926 crop	Hom. fatuoid	6	16·50±1·11	4·04±·79	—	2·50±·26	0·96±·19
	Het. fatuoid	19	33·17± ·68	4·25±·11	—	3·44±·18	1·12±·12
	Normal Kanota	8	41·88± ·30	1·27±·21	—	4·50±·21	0·87±·15
Het. fatuoid Victory, Nos. 25-98 and 25-156, 1926 crop	Hom. fatuoid	4	11·50± ·56	1·66±·40	—	2·00±·24	0·71±·17
	Het. fatuoid	72	47·24± ·43	5·40±·30	—	2·28±·09	1·14±·06
	Normal Victory	17	51·06±1·38	8·42±·98	—	2·35±·26	1·61±·19

damaged by the frit fly, as were a number of other late sown strains. As shown in Table III, they germinated fairly well, but the final survival rate was only about 35 per cent. The surviving 32 plants gave a perfect 1 : 2 : 1 ratio.

Seed from an F_1 plant of the cross Hom. fatuoid var. Sir Douglas Haig × normal Sir Douglas Haig was obtained from Mr R. J. Chittenden of the John Innes Horticultural Institution, who made the cross in 1922, and grew the F_1 in 1923. As shown in Table III, this F_2 gave a good 1 : 2 : 1 ratio. Typical panicles of the three classes of F_2 segregates of this strain, 26-24, are shown in Plate XIX.

THE CYTOLOGY OF TYPE 1 FATUOIDS.

The cytological study of the different strains of fatuoids included in Type 1 has shown that all three classes of segregates possess the normal number of chromosomes, viz. 42. In the numerous normal segregates that have been examined, the cytological conditions are more irregular

than in normal pure line varieties of *A. sativa*, though in general features
they are similar. The presence of laggard and vagabond chromosomes is
noted more frequently, and fewer cells show perfectly regular splitting
of the bivalents in the first anaphase. A wide range of variation in the
degree of irregularity is seen in different preparations, and although
some attempts have been made to tabulate the irregularities on a com-
parative basis, the results have been unsatisfactory. Probably associated
with these chromosome irregularities, is the finding that a much larger
percentage of empty pollen occurs in normal segregates of het. fatuoids
than in normal plants of pure varieties. Specific chromosome irregularities
such as the formation of trivalents or quadrivalents have not definitely
been found in normal segregates. Two plants of strain 24–21, classified
as "probable het." were studied very closely for the occurrence of
trivalents, which were expected, but only one structure of possible
trivalent nature was found. This is shown in Fig. 15. It is possibly only
a peculiar-shaped bivalent. As mentioned previously, the progeny tests
later showed these "probable het." plants to be normals.

Het. fatuoid plants of Type 1 show cytological irregularities similar
to those of normal segregates, and in addition have characteristic chromo-
some arrangements. The great majority of the pollen-mother-cells appear
to have the normal chromosome complement of twenty-one bivalents
at the heterotypic division, but occasionally one finds a trivalent and a
univalent, giving a total of 1III, 19II, and 1I. More frequently one of
these but not both are seen, and, still more frequently, a complex of
possibly but not certainly trivalent nature is observed. The chromosomes
of oats are very large in proportion to the size of the cells, and practically
always overlap more or less; they differ considerably in size and shape,
and the number is high. It is therefore not surprising that clear evidence
of the presence of a trivalent and a univalent in the same cell is found
only rarely. The number of times they can be seen probably gives very
little indication of the number of times they actually occur. When the
normal number of chromosomes is present, a trivalent must always be
accompanied by either a univalent or another trivalent, and no very
clear evidence for the latter possibility has been found, though figures
which might possibly be interpreted in that way have been seen. In
the accompanying figures trivalents and univalents are shown at various
stages, and where any doubt is felt regarding one of the structures this
is indicated. Sometimes, instead of a trivalent and univalent, a very
loosely united bivalent, Fig. 6, is seen.

At the homotypic metaphase two chromosomes are sometimes seen
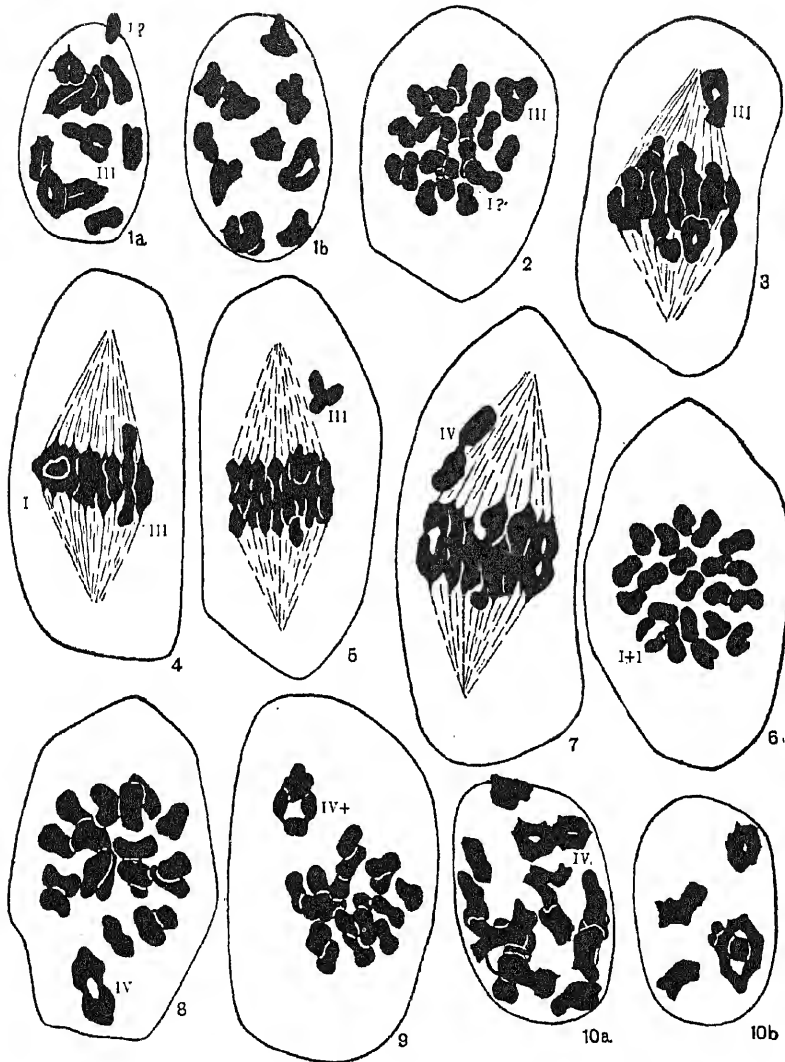
Figs. 1–6, het. fatuoids, and Figs. 7–10, hom. fatuoids from strain 24–20. For genetic results see Table I. All heterotypic divisions. Fig. 1 *a*, diakinesis, $9_{II} + 1_{III} + 1$ probable univalent displaced by knife; 1 *b*, $10_{II} + 1$ cut fragment. Fig. 2, metaphase, polar-view showing trivalent and probably $19_{II} + 1_I$. Fig. 3, side view metaphase with trivalent at pole. Fig. 4, side-view metaphase with trivalent and univalent. Fig. 5, side-view metaphase with trivalent and probable univalent. Fig. 6, polar-view metaphase with very loosely united bivalent. Fig. 7, side-view metaphase with loosely associated quadrivalent. Fig. 8, polar-view metaphase, $19_{II} + 1_{IV}$. Fig. 9, polar-view metaphase with peculiar complex, probably quadrivalent with overlapping bivalent. Fig. 10 *a*, diakinesis, $14_{II} + 1_{IV}$; 10 *b*, 5_{II}.
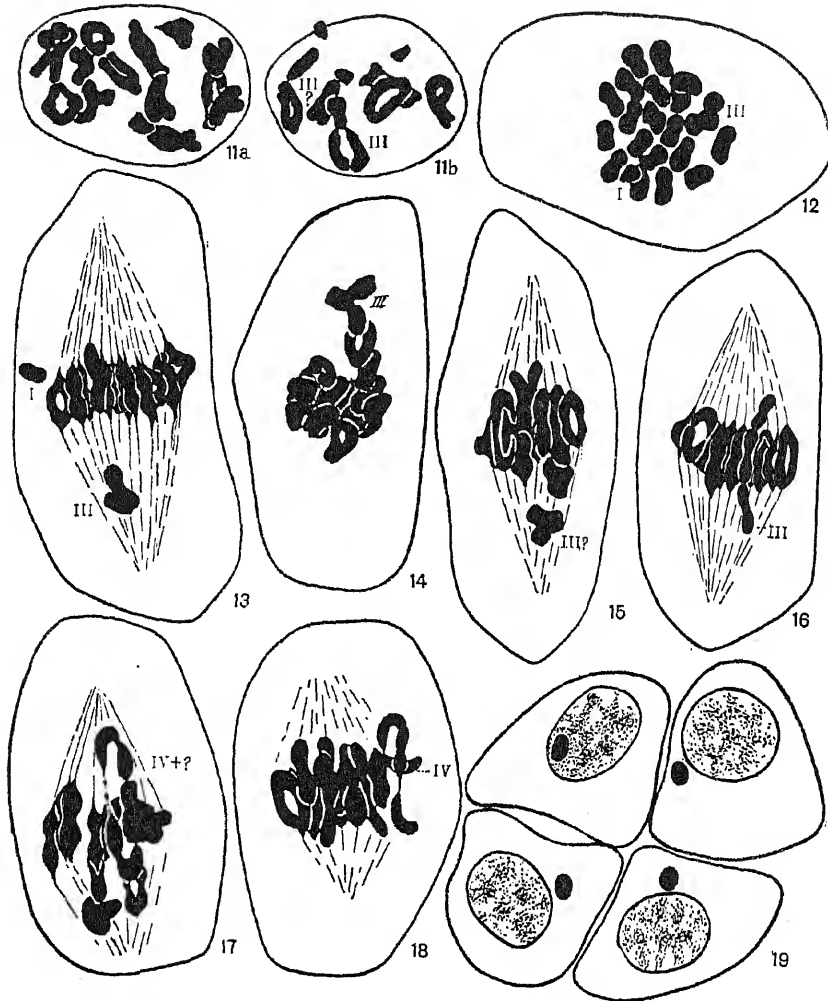
Figs. 11–18, all heterotypic divisions. Fig. 11 *a* and *b*, diakinesis, one clear and one doubtful trivalent; het. fatuoid Aurora from 25–58. Fig. 12, polar-view metaphase, $19_{II} + 1_{III} + 1_{I}$; het. fatuoid Kanota from 26–16. Fig. 13, side-view metaphase with trivalent and univalent; het. fatuoid Newmarket from 25–82. Fig. 14, late prophase with trivalent slow in approaching the plate; het. fatuoid Aurora from 25–58. Fig. 15, side view metaphase with doubtful trivalent; normal segregate from 24–21. Fig. 16, side view metaphase with trivalent; hom. fatuoid from 24–21. Fig. 17, side-view metaphase with quadrivalent or larger complex; hom. fatuoid from 24–21. Fig. 18, side-view metaphase with quadrivalent; hom. fatuoid from 24–21. Fig. 19, pollen tetrad with four chromatin bodies left out of the nuclei; hom. fatuoid from 24–1.
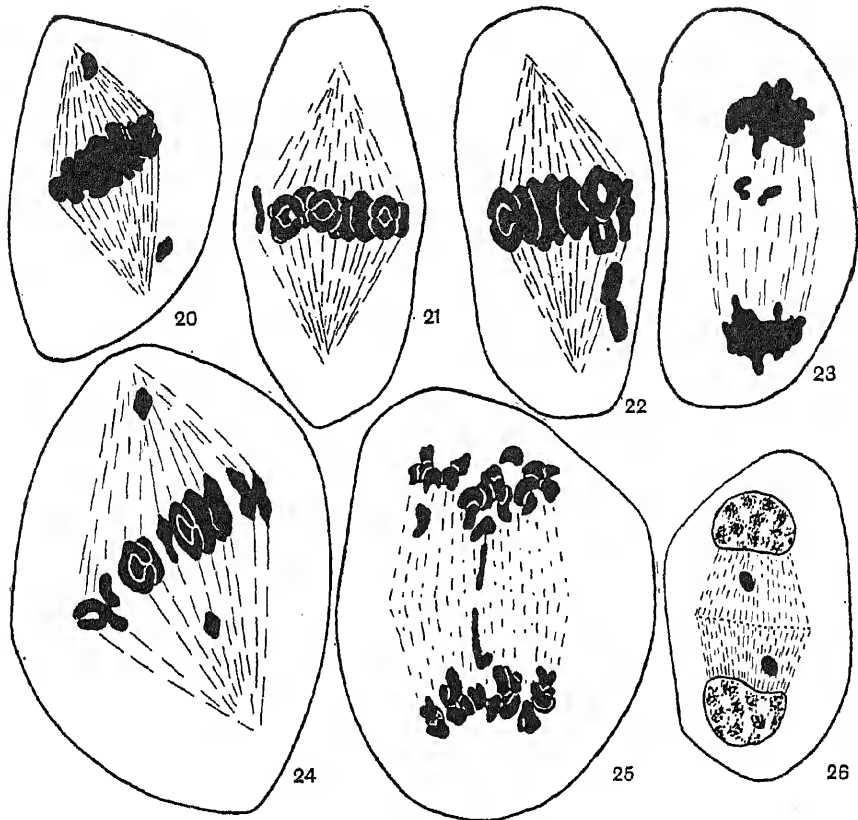
Figs. 20-24, and 26, homotypic divisions. Fig. 20, side-view metaphase with two daughter univalents at the poles; hom. fatuoid F_2 segregate 25-120. Fig. 21, side-view metaphase with daughter univalent at plate; het. fatuoid Kanota from 26-16. Fig. 22, side-view metaphase with two delayed, attached chromosomes; het. fatuoid Kanota from 26-16. Fig. 23, side-view anaphase with split univalent het. fatuoid Kanota from 26-10. Fig. 24, side-view, metaphase permanent smear preparation with daughter univalents at the poles; het. fatuoid Kanota from 26-10. Fig. 25, heterotypic anaphase, side view, permanent smear preparation with split univalent; hom. fatuoid Aurora from 25-58. Fig. 2 telophase with chromatin bodies left out of nuclei; hom. fatuoid F_2 segregate 25-120.

to be united, or at least very closely associated, instead of all being in-dependent (Fig. 22). Either two or four univalent daughter chromo-somes, presumably resulting from the splitting of unpaired chromosomes in the first division, are sometimes found towards the poles at the homo-typic metaphase, or at the plate in the anaphase, Figs. 20, 23 and 24. Where there are two such split univalents they may be both in one of the daughter cells, or one in each. Where there are four, two have always been found in each cell. Occasionally the membrane is re-formed around

the daughter nuclei before these laggards reach the poles, and they are left out in the cytoplasm of the microspores similarly to those shown in Fig. 19, which are from hom. fatuoids.

In hom. fatuoids of Type 1, besides the indefinite irregularities, one finds occasional quadrivalent and trivalent chromosome complexes. The latter occur only very rarely, and the formation of quadrivalents appears to be the characteristic feature of these hom. fatuoid segregates. Diakinesis which furnishes good evidence of trivalents is, unfortunately, not as satisfactory a stage for the detection of quadrivalents. Figure eights are seen frequently at this stage, but generally it is difficult to determine whether they are large, twisted bivalents, or quadrivalents made up of smaller chromosomes. An example of which there is little doubt is shown in Fig. 10 *a*. The number of times such figures can be detected probably gives very little indication of the number of times they actually occur. At the heterotypic metaphase quadrivalents are detected with more certainty, especially in iodine-gentian-violet preparations. In haematoxylin preparations, such as Figs. 9 and 17, the presence of overlapping chromosomes usually prevents exact interpretation, and one can only say that there are certainly not fewer than four united chromosomes, but in gentian-violet preparations the definitely quadrivalent nature can often be seen. The homotypic divisions are usually regular, but occasionally one or two split univalents are found in either one or both of the daughter cells, as in het. fatuoids described above. Microspore tetrads with the four microspores in a row, or with one pair at right angles to the other, instead of the normal bilateral arrangement, are found fairly frequently, but no supernumerary microspores have been noted.

FATUOID TYPES 2 AND 3.

The fatuoids first considered under this heading are the progeny of a single het. fatuoid panicle found by Mr J. Ficht in a head selection plot of Victory oats on the University Farm, Edmonton, in 1924. This panicle bore 54 spikelets of which 45 had twisted, geniculate awns on the lower grains, and 9 were awnless. All but three of these seeds have been sown during 1925 and 1926, under the number 24–22, but they have produced only 14 mature plants, of which 7 were normals, 6 het. fatuoids, and 1 a sterile dwarf hom. fatuoid. The awned and awnless spikelets were sown separately and there is no evidence that they were genetically different. Though the numbers are very small, this seems to be a type analogous to Nilsson-Ehle's C type speltoids which give

het. speltoids and normals in a ratio of 1 : 1, plus a few weak hom. speltoids. Typical panicles of the three classes of segregates from one of the six het. fatuoid offspring (strain 25–98) are shown in Plate XIX.

Two of the het. fatuoid progeny grown in 1925, nos. 25–98 and 25–156, and one of the normal progeny, 25–97, were propagated in 1926. Twenty-two seeds from the latter, sown 24. ii. 26, produced 22 normal progeny. Of 66 seeds from het. fatuoid 25–98 sown 22. ii. 26, and 44 seeds sown 11. iii. 26, a total of 94 germinated, but many were very slow-growing, weakly plants, and these were largely destroyed by frit, only 60 plants reaching maturity. From Tables I, II and III, it may be noted that almost perfect survival rates were obtained from sowings of Type 1 fatuoids on these dates, so that the elimination in this strain is clearly specific, and not due to date of sowing, which, of course, often does determine the degree of damage caused by frit. Owing to differences in the degree of awning, shape of base and hairiness of base on the primary grains on different panicles of some plants, and on different parts of single panicles, as in the original parent panicle, many plants could not be classified with certainty. After several independent examinations they were classified, pending a progeny test, as 3 sterile dwarf hom. fatuoids, 28 het. fatuoids, 14 probable het. fatuoids, 8 probable normals, and 7 normals. Of 110 seeds from het. fatuoid 25–156 sown 24. ii. 26, and 44 sown 11. iii. 26, a total of only 100 germinated, and of these only 66 reached maturity. These were classified as 1 sterile dwarf hom. fatuoid, 35 het. fatuoids, 20 probable het. fatuoids, 2 probable normals, and 8 normals. Combining these two progenies and classing the probable het. fatuoids with the het. fatuoids, and the probable normals with the normals, gives a total of 4 hom. fatuoid sterile dwarfs, 97 het. fatuoids, and 25 normals.

It seems that the segregation ratios of these two daughter plants are different from that of the parent, but until the complete progeny test is made, the exact ratio cannot, of course, be determined. Meanwhile, it appears probable that these are analogous to Nilsson-Ehle's B type speltoids which give het. speltoids and normals in a ratio of 4 or 5 : 1 plus a very few weak hom. speltoids. His B type speltoids also arise frequently from the 1 : 1 or C type. It is, of course, impossible to say with certainty that the extensive elimination of plants during the growth period in 1926 was selective with regard to the fatuoid character. An experiment to determine this point is being made in 1927.

In the calculations upon relative vigour of the different classes of segregates from these two plants (Table IV), the most doubtfully classified

plants have been omitted. The mean height of the normals is 3·8 inches greater than that of the het. fatuoids, but the deviation within the classes is so great that the inter-class difference is statistically of doubtful significance. The hom. fatuoids are quite dwarf and very weak. Their sterility is nearly absolute, but occasional panicles have one or two seeds developed.

The Cytology of Fatuoids of Types 2 and 3.

Cytological material collected in 1925 from het. fatuoid plant 29–98 was examined during March and April 1926 and the chromosome number was found to be only 41 instead of the normal 42. Had this interesting fact been discovered earlier, more seeds of this plant and sister het. fatuoids would, of course, have been sown in 1926. Larger sowings are being made this year. In the meiotic divisions of the pollen-mother-cells the 41 chromosomes regularly form 20 bivalents, leaving one unpaired univalent. Thirty-three cells at the heterotypic anaphase stage, examined in side view, each showed the one univalent lagging at the plate and splitting longitudinally. In 204 cells at the metaphase examined in side view, the univalent was found lying off the plate in 83 cases, while in 121 cases it could not be seen and was presumably on the plate amongst the bivalents. At telophase most cells had the normal appearance, the halves of the univalent apparently having reached the poles before the nuclear membranes were re-formed, but in a few cases these daughter-univalents were left out of the re-formed nuclei. Fig. 30 *a* and *b* shows a heterotypic anaphase in polar view. In Fig. 30 *a* there are 20 chromosomes going to one of the poles, and one univalent at the plate just splitting, this being at a much lower optical level. The 20 chromosomes going to the other pole are shown in 30 *b*. Six het. fatuoid plants of the 1926 generation from plants 25–98 and its sib 25–156 have been cytologically studied and found in general to be similar to 25–98 described above. Fig. 32, a side view of the first metaphase in an embryosac-mother-cell, shows a univalent chromosome on the plate. The number of bivalents cannot here be counted. In the homotypic anaphase there is usually one daughter univalent lagging at the plate in each of the daughter cells, as shown in Fig. 31 *a* and *b*. Occasionally both are in one cell. The behaviour so far described is, of course, such as would be expected in any case where an unpaired chromosome is present. In the first plant studied, 25–98, no deviations from this behaviour have been observed. But in these het. fatuoid offspring an occasional unexpected feature has been found. Of 122 first anaphase figures seen in side view,
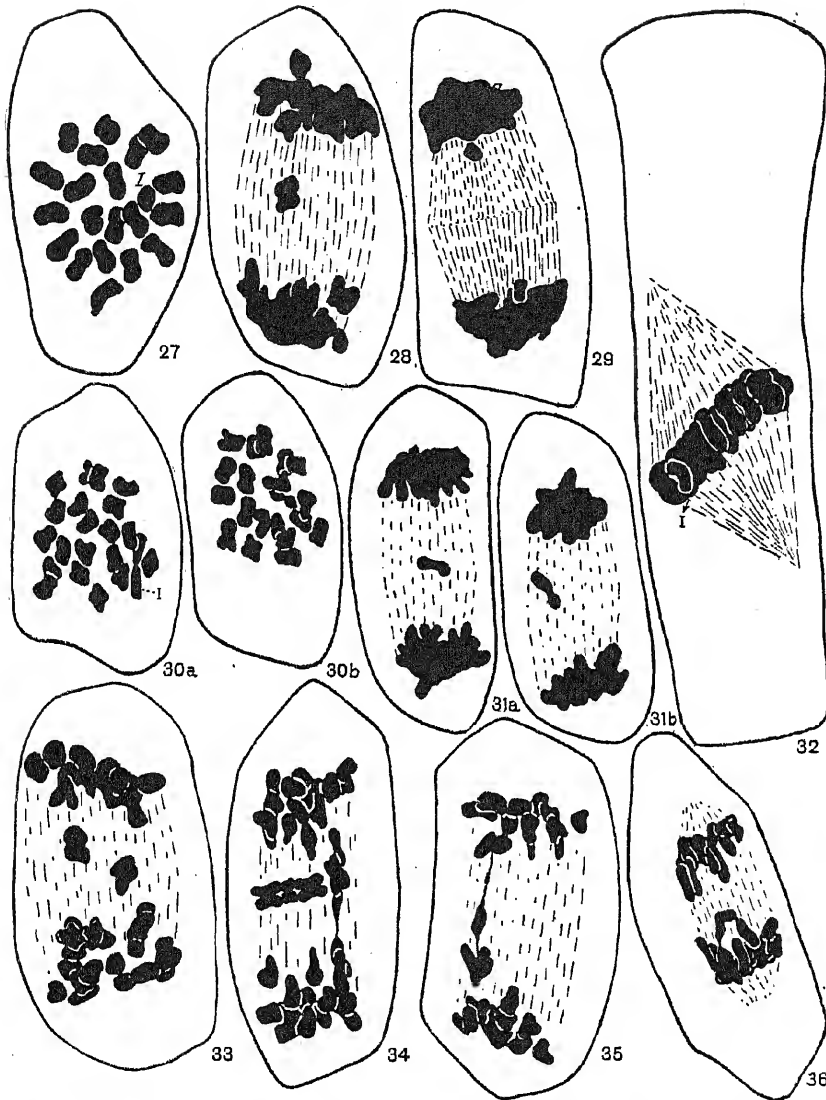
Figs. 27–30, and 32–35, heterotypic divisions; Type 3 het. fatuoids. Fig. 27, polar-view metaphase 20II + 1I; from 25–98. Fig. 28, side-view anaphase with univalent splitting at the plate; 25–98. Fig. 29, side-view telophase with split univalent; 25–98. Fig. 30 a, polar view anaphase with 20 chromosomes towards the pole and univalent at plate; 30 b, other pole with 20 chromosomes; 25–98. Fig. 31 a and b, homotypic anaphases with a daughter univalent in each cell; from 25–98. Fig. 32, embryosac metaphase showing univalent; from 25–98. Fig. 33, side-view anaphase with two univalents or one univalent and one delayed bivalent; from 25–98. Fig. 34, side view anaphase with 3 univalents and delayed bivalents; from 25–156. Fig. 35, side-view anaphase with trivalent; from 25–98. Fig. 36, homotypic anaphase, side-view, with daughter trivalent; from 25–156. See text-pages 336 and 338 for discussion of these figures.

118 show one laggard univalent at the plate, while four show other conditions less easy to interpret. The first of these, Fig. 33, seems to have two univalents, though one is rather large, and may possibly be a small bivalent. Fig. 35 shows what is almost certainly a trivalent just splitting. The third and fourth each have three laggard univalents. One of them, Fig. 34, has also several bivalents which are slow in separating. One deviation from the expected feature of two daughter univalents has been found at the homotypic anaphase. This, shown in Fig. 36, consists of a trivalent which appears to be going entire to the one pole.

The formation of the pollen tetrads does not appear to be affected greatly by the absence of one chromosome. The large majority are regular in form, though a number show small dark bodies outside their nuclei, similar to those in Fig. 19, which are presumably split univalents that have failed to reach the poles before the nuclear membranes were re-formed. The percentage of empty pollen varies very greatly in different flowers. Nine counts gave from 15 per cent. to about 75 per cent. empty. The four largest and most reliable counts are given in Table V.

In the sterile dwarf hom. fatuoids the cytological conditions are extremely irregular. At diakinesis the pairing is usually very faulty. It is, in consequence, extremely difficult to get reliable counts. Fig. 38 shows the most regular diakinesis that has been found, and it apparently has 20 bivalents, though in several cases the members of these are only very loosely united and at one end only. In hundreds of cells examined, no regular metaphase plates have been found. The nearest approach to one yet seen is shown in Fig. 39. Here there are fairly clearly 18 bivalents and four univalents. Fig. 40 shows a fairly common condition in which some of the chromosomes have formed a resting nucleus, while others remain scattered throughout the cell. The homotypic division seems frequently to be suppressed entirely. When it does occur, it is hopelessly irregular. Fig. 37 *a* and *b* shows successive sections which can only be interpreted as a homotypic metaphase in a cell which has failed to form a wall between the nuclei after the first division. The total chromosome number is probably 40 in this cell. Such divisions as this doubtless give rise to the giant pollen grains which are occasionally formed (Fig. 43). Practically no regular pollen tetrads are formed. Dyads and triads are very common, and all sorts of irregular arrangements up to octads have been found (Figs. 41 and 42). The pollen is apparently almost entirely sterile, most of the grains being completely shrunken (Plate XXI, fig. 6).

The normal segregates from the 41-chromosome het. fatuoids have the
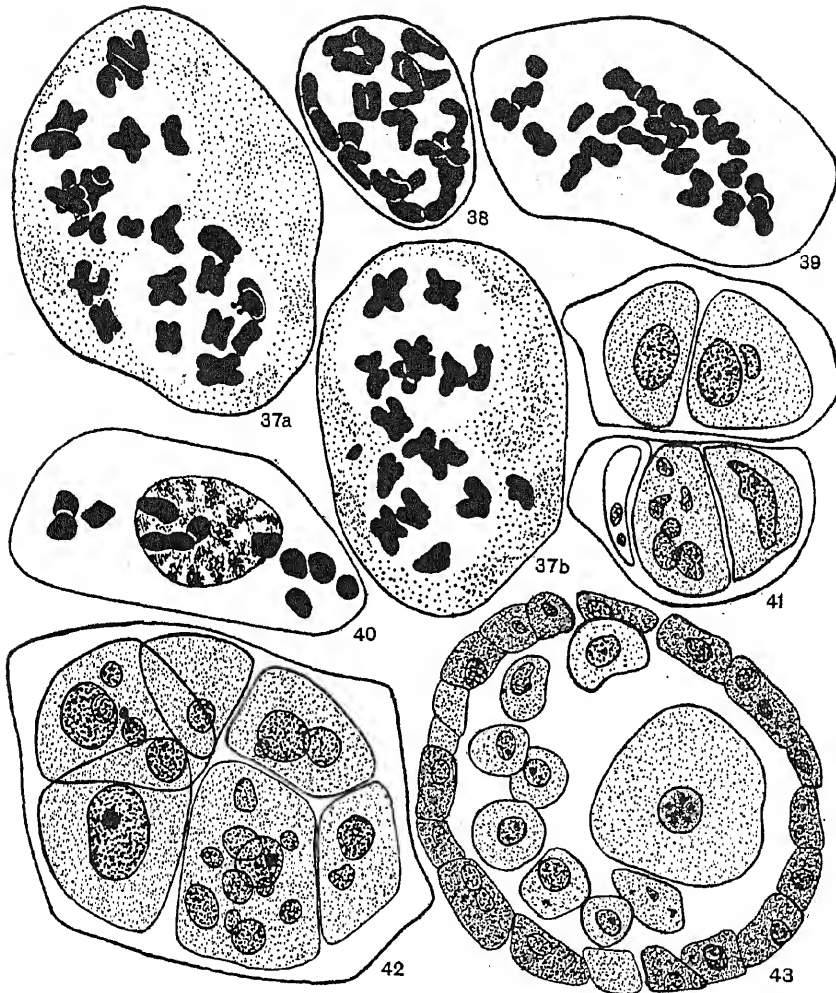
Figs. 37–43, sterile dwarf hom. fatuoids of Type 3. Fig. 37 a and b, homotypic metaphase of cell which has not developed a dividing wall after the first division. Fig. 38, diakinesis, 20π. Fig. 39, abnormal metaphase 18π+4ı. Fig. 40, abnormal heterotypic division. Fig. 41, pollen pentad, aceto-carmine preparation, 10x ocular. Fig. 42, pollen hexad, aceto-carmine preparation 15x ocular. Fig. 43, anther loculus with normal size and giant pollen grains, 5x ocular. See text-page 338 for discussion of these figures.

normal 42 chromosomes, and are as regular in their cytological behaviour as the normal segregates from Type 1 het. fatuoids.

Seeds of various types of fatuoids obtained from Dr C. H. Goulden, Winnipeg, Canada, were sown rather too late in 1926, and were all badly damaged by frit. Of 231 seeds from his Type 2 het. fatuoids, 218 germinated but only 97 remained at the end of the season, and most of these were very badly damaged by frit and secondary disease infections.

These surviving plants were classified as 3 sterile dwarf hom. fatuoids, 57 het. fatuoids, and 37 normals.

Cytological material was collected from five plants, but, presumably owing to their unhealthy condition, most of it has proved unsatisfactory. Two of the het. fatuoid plants apparently have 41 chromosomes, and are probably similar in other respects to the het. fatuoids described above, but further material must be collected and studied this year, before this can be stated definitely.

FATUOIDS OF TYPE 4.

Type 4 is so far represented by a single strain from the variety Kanota, which was obtained early in 1926 from Professor J. H. Parker, Manhattan, Kansas, together with the Type 1 het. fatuoid Kanota strains already described. The hom. fatuoid segregates from this strain are all dwarf and sterile. A number of segregates are quite awnless, and clearly normals, but others which are probably het. fatuoid show considerable variation in the degree of awning. It remains to be seen whether this is merely fluctuation, or whether this class of segregates includes two or more genetically different types. The genetic data on this strain are very limited. Of the 44 seeds sown in 1926, 35 germinated and produced 8 hom. fatuoids, 7 het. fatuoids, 12 probable het. fatuoids, and 8 normals. A single seed was obtained from one artificially selfed dwarf hom. fatuoid, and another by artificial pollination with pollen from a normal segregate. These were the only seeds produced by the 8 hom. fatuoids.

The calculations on relative vigour (Table IV) show differences between the classes of segregates which are clearly significant, despite the small size of the samples. Only 6 hom. fatuoid plants appear in these calculations, as two were killed in taking cytological material from them. In crowded plantings these hom. fatuoids would probably perish at an early stage. In the widely spaced plantings here used they produced two or three short weak culms, average 2·5, and attained an average height of 16·5 inches. The het. fatuoids and ·probable het.

fatuoids considered together attained an average height of 33·17 ± 0·68 inches, as compared with 41·88 ± 0·30 inches reached by the normal segregates. The average number of culms is 3·44 ± 0·18 in the het. fatuoids, against 4·5 ± 0·21 in the normals. The standard deviation of the height is much greater in both the hom. and het. fatuoids than in the normals.

THE CYTOLOGY OF TYPE 4 FATUOIDS.

The cytology of this type is of particular interest. Two of the hom. fatuoid dwarfs have been found to possess 44 chromosomes instead of the normal 42. In diakinesis these are sometimes seen to be arranged as 20II + 1IV, but in other cells there appear to be 22 distinct pairs, (Fig. 49). In the material so far collected, all of which has been examined, the chromosomes seem to be very irregular in their rate of approaching the metaphase plate, and are usually seen scattered throughout the cell. In many cells at the heterotypic metaphase one can be quite sure that there are more than 42 chromosomes present, but exact counts suitable for reproduction have not been found at this stage. Nevertheless, considering the circumstances, the first division proceeds more regularly than might be expected. In many cells odd bivalents lie at the poles while the remainder of the chromosomes are dividing, and in others bivalents lag at the plate during the anaphase, but these irregularities are found also in hom. and het. fatuoids of Type 1 with the normal number of chromosomes, though probably not as frequently. These irregularities make it difficult to obtain counts at the anaphase also, but Fig. 48 *a* and *b* shows a cell which has 22 chromosomes fairly clearly at each pole in the heterotypic anaphase, plus a small cut fragment in one section. This is from a preparation fixed in Carnoy's fluid, which accounts for the relatively smaller size of the chromosomes. The homotypic divisions seem to proceed fairly regularly except for the frequent occurrence of laggard chromosomes at both the metaphase and anaphase, but sufficient material was not available for an extensive study to be made of these second division stages.

The pollen tetrads formed are fairly regular in appearance, and supernumerary microspores have not yet been observed. The pollen grains up to a fairly mature stage also appear to be normal with dense cytoplasm. It appears, however, that as the pollen is ripening, degenerative processes set in. This stage has not been studied in sufficient detail for final conclusions to be drawn, but examinations of pollen from three fully ripe anthers from different plants have shown all the grains to be
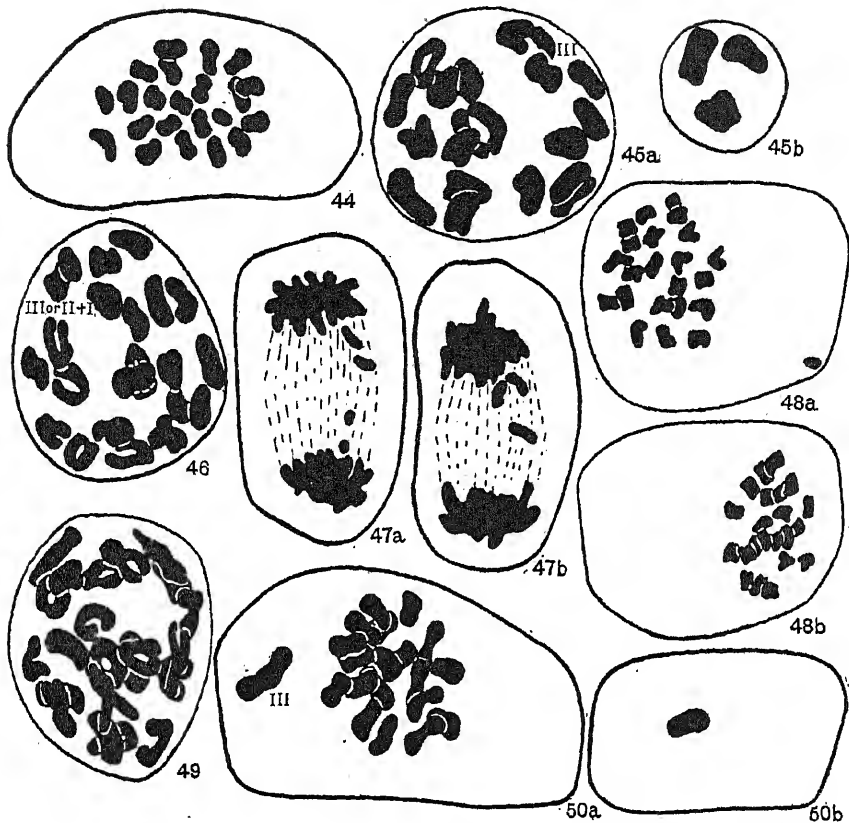
Type 4, het. fatuoids; Figs. 44–46, and 48–50 heterotypic divisions. Fig. 44, polar view metaphase, 21II + 1I; het. fatuoid. Fig. 45 a, diakinesis, 17II + 1III; 45 b, 3II; het. fatuoid. Fig. 46, diakinesis, 20II + 1III; or 21II + 1I; het. fatuoid. Fig. 47 a and b, homotypic anaphase with three daughter univalents in each cell, one of which has divided a second time; het. fatuoid. Fig. 48 a, polar-view anaphase, with 22 chromosomes plus a cut fragment; 48 b, showing 22 chromosomes at the other pole; hom. fatuoid. Fig. 49, diakinesis, 22II; hom. fatuoid. Fig. 50 a, polar view metaphase, 19II + 1III; 50 b, 1III near pole; het. fatuoid. See text-pages 341–343 for discussion of these figures.

empty, whereas in four nearly mature anthers pollen grains were found in all stages from completely dense to empty similar to those shown in Plate XXI, fig. 8.

Panicles from three het. fatuoid plants of this strain were fixed for cytological study, and all this material has been examined. One plant gave no division stages suitable for counting. Each of the other two has 43 chromosomes. Fig. 45 a and b shows a chromosome complement of 20II + 1III at diakinesis, and this seems to be the usual arrangement.

The trivalent in this figure is well in the centre of the cell, away from the cut surfaces. In Fig. 46 the extra chromosome lies on top of a bivalent, and may or may not actually be connected to it. Fig. 44 shows a metaphase plate, Carnoy preparation, in which there are clearly $21_{II} + 1_{I}$. Fig. 50 a and b shows a cell at metaphase with a clearly trivalent body well off the plate. The remainder cannot be counted with certainty, but there appear to be 19 in the one group at the plate, while another bivalent has lagged at the pole and so comes in the next section. Side views of the first anaphase in some cells show a univalent splitting at the plate while the remainder of the chromosomes have proceeded regularly to the poles. In other cells the anaphase is very irregular. No good polar views giving clear counts at the anaphase have been found. The homotypic divisions usually proceed fairly regularly except that from one to three daughter univalent chromosomes are frequently seen in each daughter cell. Fig. 47 a and b show a homotypic anaphase pair with three in each cell. In one of these cells one of the daughter univalents has divided. As shown in Table V, there is a considerably greater percentage of empty pollen in the het. fatuoid segregates of this strain than in the normals.

Cytological material was collected from two normal segregates of this

TABLE V.

Pollen Examinations.

Strain	Type of segregate	Count No. 1 Total No. of grains	Count No. 1 Percentage empty	Count No. 2 Total No. of grains	Count No. 2 Percentage empty	Count No. 3 Total No. of grains	Count No. 3 Percentage empty	Count No. 4 Total No. of grains	Count No. 4 Percentage empty	Totals Total No. of grains	Totals Percentage empty
A. fatua	—	503	0·60	439	1·59	599	0·16	—	—	1541	0·71
A. sativa var. Banner	—	309	3·24	508	4·92	413	6·78	—	—	1230	5·12
Het. fatuoid Banner, No. 24–20	Hom. fatuoid	1735	25·65	1094	14·71	775	11·48	—	—	3604	19·28
,, ,,	Het. fatuoid	1581	8·92	759	9·22	650	12·77	533	12·19	3523	10·19
,, ,,	Normal Banner	1827	10·83	1420	8·24	1728	14·06	—	—	4975	11·21
Hom. fatuoid × Banner, Nos. 25–29 and 25–46	Hom. fatuoid	1557	14·85	377	11·41	987	19·65	—	—	2921	16·02
,, ,,	Het. fatuoid	183	19·12	2376	17·21	878	18·91	—	—	3437	17·75
,, ,,	Normal Banner	565	13·27	2022	3·21	465	10·54	—	—	3052	6·19
Het. fatuoid, Kanota, No. 26–13	Hom. fatuoid	600	100·00	530	100·00	500	100·00	344	77·03*	1630	100·00
,, ,,	Het. fatuoid	94	7·45	1154	9·78	648	11·42	—	—	1887	10·23
,, ,,	Normal Kanota	814	4·18	1010	4·85	706	2·27	—	—	2530	3·91
Het. fatuoid Victory, No. 24–22	Hom. fatuoid	x	100·00	x	100·00	x	100·00	—	—	x	100·00
,, ,,	Het. fatuoid	224	32·70	567	25·93	518	20·46	690	47·83	1999	32·72
,, ,,	Normal Victory	1226	8·93	1520	14·74	—	—	—	—	2746	12·15

* Slightly immature, not included in total.

strain. Both have the normal chromosome number, 42, and the divisions are comparatively regular. The pollen in three flowers examined was particularly good, as shown in Table V.

It is obvious that a much more extensive study, both genetical and cytological, must be made of this very interesting strain of fatuoids, but the evidence from the limited number of plants studied seems to be fairly clear so far as it goes.

Miscellaneous Hom. Fatuoids.

A large number of strains of hom. fatuoids obtained from various sources have been propagated. They are recorded in Table VI. All but two have bred true to the hom. fatuoid type. The het. fatuoid from hom. fatuoid Newmarket may have arisen through natural crossing, as pollination was not controlled. The hom. fatuoid Banner which has given a normal plant among its progeny, was artificially selfed. Even had it not been controlled, natural crossing with a normal plant would be expected to produce only the het. fatuoid and not the completely normal type direct from the hom. fatuoid. There is, of course, the possibility of accidental admixture of seed, but in view of the particular circumstances I consider this very improbable. Further evidence may be obtained from 1927 sowings, and until this is available the possibility of this normal plant having arisen as a bud sport need not be considered.

One hom. fatuoid strain, Banner type, 24–17, gave amongst its progeny one sterile, dwarf hom. fatuoid. Its cytological behaviour is similar to that of the hom. fatuoid sterile dwarfs from Type 3, which have been described. Its chromosome number is also almost certainly 40. This plant bore six viable seeds, four of which were on panicles which were artificially selfed, and two on uncontrolled panicles. All six seeds produced sterile dwarf hom. fatuoid plants like the parent.

Many plants of the hom. fatuoid strains Banner 24–1, Storm King 25–21, Old Island 25–22, and Newmarket 25–23, have been cytologically examined. Most of the findings in the first three of these strains have been reported in a previous publication (1926). They are in all essential particulars similar to the hom. fatuoid segregates of Type 1 here described, but the irregularities seem to occur less frequently in these strains, which have been breeding true to type for some years, than in the newly segregated hom. fatuoids.

One hom. fatuoid plant from the Victory strain 26–21, which, as previously mentioned, originated directly from the normal in Mr C. V. B. Marquand's cultures, has also been examined cytologically. It has the

TABLE VI.

Miscellaneous Homozygous fatuoids.

Strain and Origin	Strain number	Uncontrolled or selfed	Year sown	Number of seeds	Progeny			
					Homozygous fatuoid	Heterozygous fatuoid	Normal	Total
Hom. fatuoid Banner type; selected from classroom material, University of Alberta	24-1-7	S.	1925	100	All	—	—	—
„ „ „ ,	„	S.	1926	88	76	—	—	76
Hom. fatuoid Old Island; from N. Criddle, Ottawa	25-22	U.	1925	10	2	—	—	2
„ „ „ „	25-25	U.	1925	10	10	—	—	10
„ „ „ „		U.	1926	11	8	—	—	8
„ „ 25-22 „	25-75	S.	1926	11	10	—	—	10
Hom. fatuoid Storm King; from N. Criddle, Ottawa	25-21	U.	1925	20	5	—	—	5
„ „ „ „	25-26	U.	1925	10	6	—	—	6
„ „ „ „		U.	1926	11	6	—	—	6
„ „ 25-21 „	25-76	S.	1926	11	10	—	—	10
Hom. fatuoid Newmarket; from N. Criddle, Ottawa	25-23	U.	1925	20	16	1	—	17
„ „ 25-23	25-83	S.	1926	11	10	—	—	10
„ „ „	25-84	U.	1926	33	30	—	—	30
Hom. fatuoid Victory; selected from variety plot, Edmonton, Alberta	24-23	U.	1925	10	9	—	—	9
„ „ „		U.	1926	11	10	—	—	10
„ „ from 24-23 „	25-87	S.	1926	11	11	—	—	11
Hom. fatuoid Banner; from N. Linden, Wetaskiwin, Alberta	24-28	U.	1925	10	9	—	—	9
„ „ „		U.	1926	11	11	—	—	11
„ „ 24-28 „	25-88	S.	1926	11	11	—	—	11
Hom. fatuoid Banner; from N. Linden, Wetaskiwin, Alberta	24-29	U.	1925	10	7	—	—	7
„ „ „		U.	1926	6	5	—	—	5
„ „ 24-29 „	25-94	S.	1926	11	9	—	1	10
Hom. fatuoid, Banner type; collected on roadside, Edmonton	24-17	U.	1925	10	*9	—	—	9
„ „ from 24-17	25-95	S.	1926	11	11	—	—	11
„ „ dwarf from 24-17	25-96 a	S.	1926	4	†4	—	—	4
„ „ „ „	25-96 b	U.	1926	4	†2	—	—	2
Hom. fatuoid Banner; selected from variety plot, Edmonton	24-24	U.	1925	10	All	—	—	—
„ „ from 24-24	25-86	S.	1926	33	30	—	—	30
Hom. fatuoid Supreme; from C. V. B. Marquand, Aberystwyth	26-26	U.	1926	11	10	—	—	10
Hom. fatuoid Black Winter; from C. V. B. Marquand, Aberystwyth	26-30	U.	1926	11	10	—	—	10
Hom. fatuoid Victory; from C. V. B. Marquand, Aberystwyth	26-31	U.	1926	11	8	—	—	8
Hom. fatuoid Victory; from R. J. Garber, Morgantown, Virginia	25-53	U.	1926	11	8	—	—	8
Hom. fatuoid Garton 784 from R. J. Garber, Morgantown, Virginia	25-56	U.	1926	11	6	—	—	6
Hom. fatuoid Aurora; from R. J. Garber, Morgantown, Virginia	25-61	U.	1926	11	11	—	—	11
Hom. fatuoid Sir Douglas Haig; from R. J. Chittenden, Merton	26-2	U.	1926	22	18	—	—	18
F_2 Hom. fat. Sir D. Haig × Hom. fat. Sir D. Haig; from R. J. Chittenden	26-21	U.	1926	11	8	—	—	8
Hom. fatuoid Siberian; from Prof. Summerby, Ste Anne, Quebec	25-160	U.	1926	14	1	—	—	1
Hom. fatuoid Kanota; from J. H. Parker, Manhattan, Kansas	26-1	U.	1926	11	1	—	—	1

* One plant was sterile, dwarf hom. fatuoid, bearing only six viable seeds.
† Dwarf sterile hom. fatuoids similar to the parent.

normal number of chromosomes and cytological behaviour similar to that of these other hom. fatuoids.

Two of these strains of hom. fatuoids, 24–1 and 26–2, have given Type 1 het. fatuoid strains 25–29, 25–46, and 26–24 when crossed with normal oats. In view of this and of the cytological characteristics of all of the many plants investigated one seems justified, for the present at least, in including under Type 1 all hom. fatuoids which are comparatively fertile and of normal vigour for their variety. Hom. fatuoids of the other types so far obtained are, as has been shown, quite distinct in these respects.

OTHER MISCELLANEOUS FATUOID TYPES.

Three typical hom. fatuoid seeds from the variety Abundance, obtained from Mr C. W. Leggatt, Calgary, were planted in 1926, and have given two ordinary hom. fatuoid plants, and one which is intermediate in character. All the grains of the latter bear twisted geniculate awns as hom. fatuoids do, but in the shape of the bases and the degree of hairiness they are similar to het. fatuoids.

A somewhat similar intermediate type from the variety Ceirch du bach was obtained from Mr E. T. Jones, Aberystwyth. He had previously found it to breed true with respect to these "semi-fatuoid" characters. Twenty-two seeds, sown in 1926, gave 21 plants, all similar in grain type to the parent, but one was very weakly and dwarf. Cytological material has been collected from these plants, but not yet studied.

A fatuoid type has recently been found in *A. ludoviciana*.

DISCUSSION.

The correlations found between the chromosome numbers and cytological behaviour on the one hand, and the genetic ratios and relative vigour of the different classes of segregates from het. fatuoids on the other, are so close that one is forced to assume a causal relationship. While the data are not yet quite extensive enough to permit the formulation of a complete scheme covering all the types of fatuoids known, they do appear to indicate clearly the general nature of the mechanism which produces fatuoids from normal cultivated oats.

The different types of fatuoids here described, and those of Goulden (1926) bring our genetic knowledge of fatuoids very closely into line with that of speltoids. Similarly, Winge (1924) found cytological conditions in a 1 : 2 : 1 type strain of speltoids practically identical with those here described in the analogous type of fatuoids. But, on the other

hand, in a 1 : 1 type speltoid and in a dwarf club type he found normal cytological conditions, whereas, in the more or less analogous fatuoid forms here described, very irregular cytological conditions prevail. In other forms of wheat "aberranten" not duplicated in this fatuoid series, he found some with abnormal chromosome numbers and behaviour. From this evidence Winge concludes that speltoids, and the other mutant forms of wheat studied, arise through chromosome aberrations. To distinguish them from point or gene mutations he designates them "aberranten." Winge mentions that Lindhard (1922) had, without cytological evidence, suggested the possibility of speltoids arising through chromosome aberration, but points out that most other workers have sought explanations in various hypotheses of gene mutation complicated by heterogamy and differential elimination of zygotes in certain cases.

The earlier literature concerning the origin of fatuoids is much less complicated than that on speltoids, since most workers with the former have found only the 1 : 2 : 1 segregation type, here designated Type 1, whereas the more peculiar segregation types of speltoids have long been known. The exponents of natural crossing hypotheses have been more numerous in the literature of fatuoids than of speltoids. The more recent work on fatuoids has, however, shown a striking similarity to that on speltoids. Since the hypothesis of natural crossing between *Avena sativa* and *A. fatua* has been shown to be inadequate to explain the origin of fatuoids, most workers have favoured a gene mutation hypothesis. So long as only Type 1 strains of fatuoids were known, and no splitting of the group of characters comprising the fatuoid complex had been observed, this hypothesis gave a satisfactory explanation. Garber (1922) and most other workers have believed that the characters which distinguish fatuoids are conditioned by a single factor difference, though Nilsson-Ehle (1921 a) favoured the assumption that a very closely linked group of factors rather than a single factor is involved. Mr E. T. Jones' discovery of a true-breeding intermediate type fatuoid in Ceirch du bach, and the discovery here described of a fully awned but intermediate-base type arising from hom. fatuoid Abundance, prove the validity of Nilsson-Ehle's assumption, and, of course, in themselves indicate that more than a single gene mutation is involved in the production of fatuoids.

In a short note on the cytology of one strain of hom. fatuoids (Huskins, 1925) it was pointed out that the evidence warranted the hypothesis that fatuoids arise through chromosome aberration. The genetic evidence now available suggests that either a whole chromosome or a sectional change, rather than a single gene mutation is involved. The

cytological evidence seems clearly to show that it is whole chromosome changes which produce the different types of fatuoids here studied. The true-breeding intermediate fatuoid forms may be found to owe their origin to either gene or "deficiency" mutations, but crossing-over between "semi-homologous" chromosomes would seem to be an equally valid possible cause of origin.

Before proceeding to outline a diagrammatic scheme which is believed to be capable of explaining the main general features involved in the origin of fatuoids and speltoids it is necessary to consider some of the evidence on the origin and genetic nature of cultivated wheat and oats. Simple gene differences appear to be inadequate to explain many of the most interesting facts discovered by hybridisation studies in wheat and oats as well as by studies on speltoids and fatuoids. Deviations from ratios of the types produced by the segregation of simple factors or genes may be expected when one is dealing with polyploid species, especially if of mixed ancestry. The consideration of such ancestry is therefore of importance, even though only generalised ideas are yet possible. The origin of cultivated wheat and oats has been much discussed by Percival (1921), Gates (1924), Vavilov (1926), and many others. Only a few general principles can be discussed here.

Percival (1921), p. 355, has pointed out that mutations occur much more commonly in *Triticum vulgare* or *T. compactum*, which he considers to be of hybrid origin, than in *T. durum* or *T. dicoccum*, and he states that he has never known a mutation in *T. monococcum*. We now know that the first two of these species are hexaploid, the third and fourth tetraploid and the last diploid. Winge (1924) has not especially considered the relation of the probable hybrid origin of cultivated wheats to the speltoid problem, but has stressed the polyploid nature of these species.

Probably hybridisation and polyploidy are related phenomena in the origin of wheat and oats, and are equally and jointly responsible for the origin of speltoids and fatuoids as well as for many of the peculiar features discovered in hybridisation studies.

In both *Triticum* and *Avena* the commonest cultivated species are phylogenetically hexaploid. Several tetraploid *Triticum* species are fairly widely cultivated, but tetraploid oats are comparatively rare. In both genera the diploid species are of very low economic value. In the hexaploid species of both genera, multiple independent factor series have been found. The best-known case is Nilsson-Ehle's discovery that different varieties of wheat carry one, two or three independent factors for the red colour of the grain. Boshnakian (1922) states: "There exists

in wheat more than one density factor belonging to multiple series."
Åkerman (1924) has found varieties of oats with either one or two indepen-
dent factors for black glume colour, and his findings in oats which bleach
in direct sunlight (1922) fit a triple independent factor scheme.

The present polyploid series in *Triticum* and *Avena* might, of course,
have arisen either by multiplication or reduction of chromosome numbers,
but all the available evidence favours the former possibility. The multi-
plication may have occurred directly, or through the intervention of
hybridisation, or by a combination of the two. One primitive diploid
species may have formed a tetraploid by direct doubling, or a tetraploid
may have arisen from the crossing of two diploid species. The hexaploid
species almost certainly arose through the hybridisation of a tetraploid
with another diploid species, as Clausen and Goodspeed (1925) have
demonstrated experimentally in *Nicotiana*.

This conception of a hybrid polyploid ancestry for wheat and oats
is based primarily on the evidence available from geographical distribu-
tion and taxonomic studies, and more definitely on the basis of analogy
with experimental evidence from other genera such as Karpechenko's
(1927) artificial species from the cross *Raphanus sativus* × *Brassica
oleracea*, the fertile octoploid *Aegilotricum* species obtained by Tschermak
and Bleier (1926) from the crosses *A. ovata* × *T. dicoccoides* and *A. ovata*
× *T. durum* and the case of *Primula kewensis*. It fits the extensive
genetic and cytological evidence from intervarietal, interspecific, and
intergeneric crosses in the cereals, as well as the speltoid and fatuoid
data, much better than that of the direct triplication of a single diploid
set of chromosomes assumed by many workers. Such a triplication with-
out the intervention of hybridisation has never been shown to occur in
experimental studies. If one assumes it, then one has further to assume
that various parallel and complex mutations must have since occurred
in different tetraploid and hexaploid species.

Direct reconstruction of modern wheat or oat species by hybridisa-
tion of existing primitive species may be an impossible task, and so
direct proof or refutation of this conception may never be available.
But evidence strongly corroborative of its plausibility is obtained from
the artificial species mentioned. The case of *Primula kewensis* as
demonstrated by Newton and Pellew (1926) is probably one of the best
instances on account of the correlated genetic and cytological evidence.
The cross *P. verticillata* × *P. floribunda* produces *P. kewensis*. Both the
parents are diploid species having 9/18 chromosomes. The immediate
offspring is a sterile diploid. The striking feature of its cytology is that

practically perfect pairing occurs between the chromosomes of the two species, and yet few if any viable gametes are formed. By bud-sporting, fertile forms of *P. kewensis* have several times been obtained. These have been found to be tetraploids, having 18/36 chromosomes. These tetraploids breed relatively true to type except with regard to mealiness. The cytological behaviour is well in accord with these genetic facts, as the usual arrangement of the chromosomes at the metaphase is $16_{II} + 1_{IV}$. If the factors for mealiness and non-mealiness are carried by the chromosomes constituting this quadrivalent, then the random distribution of the members of this group will account for the segregation in respect to mealiness. The correct pairing and separation of the other chromosomes will account for the lack of segregation of other characters, and so this known hybrid, with specific characters which exist only as the expression of a balance between different sets of factors, breeds relatively true to type. The exceptions other than mealiness have all been found to be due to chromosome aberrations.

Applying this conception of hybrid polyploid nature to cultivated oats, it appears that all the known facts are in conformity with it. Unfortunately, less is known of both the origin and the genetic nature of the different oat species, than is known of wheat. Vavilov (1926) shows that cultivated oats have a polyphyletic origin, and that they were known to the ancients as weeds admixed with other cereals.

A large number of hexaploid types of oats, many of which are of widely different geographical distribution, have been given specific rank, but with few exceptions these species intercross and produce offspring with undiminished fertility. The characters on which their specific identity is based are in many instances found to be transmitted similarly to a single Mendelian factor difference. The cross *A. fatua* × *A. sativa*, which is the one most widely studied, is an excellent example. Surface (1916) found eight characters of base, awn, and pubescence to be linked, and to segregate in a 1 : 2 : 1 ratio. In the cross *A. nuda* × *A. sativa*, Caporn (1918) found that the F_1 plants carry both *nuda* and *sativa* type grains. The F_2 shows a bewildering mixture of forms, but the plants with only *sativa* type grains occur in a ratio of 3 : 1 of the other types. The most interesting fact which emerges is that the multiflorous condition of the spikelets appears only when the grains are of the *nuda* type. Love and McRostie (1919) from a similar cross, found in the F_2 a 1 : 2 : 1 ratio of *sativa*, intermediate, and *nuda* forms. The intermediate segregates varied greatly in appearance, but in general all tended to give a 1 : 2 : 1 ratio, while those of the parental types bred true with

respect to the characters under consideration. In these crosses it seems reasonable to assume that the parental species differ in their specific essentials by a single pair of chromosomes carrying the factors responsible for the production of these linked characters. Some preliminary observations indicate that there is probably cytological evidence for this assumption. Such an assumption further explains the very rare occurrence of crossing-over or the splitting of these factor complexes.

Professor Shegalov of Moscow has probably the largest range of oat hybrids in the world, but unfortunately very few of his results have yet been published. A few of his findings, personally communicated, may with his permission be mentioned. In any cross of *A. sativa* × any wild species, he always obtains a few *A. nuda* plants in later generations. The cross *A. brevis* × *A. nuda biaristata*, both diploid species, gives an F_1 which is like *A: strigosa*, a third diploid species. The characters of *A. sterilis* are dominant to those of *A. fatua* and recessive to those of *A. sativa*, but in neither case completely so. *A. sterilis* × *A. sativa* gives a monohybrid segregation. *A. sterilis* × *A. fatua* gives a few intermediate forms in the second generation.

All these facts seem to support the hypothesis that cultivated oats have arisen from hybridisation between two or three primitive species, followed by polyploidy.

Malinowski (1926) has made an ingenious attempt to explain certain data from interspecific crosses in *Triticum* by assuming that the chromosomes assort in united groups of threes in hexaploid wheat species, and in twos in tetraploids, instead of individually. While this would explain some phenomena it not only lacks cytological evidence but is in contradiction to that so far available. If we follow the conception of a hybrid polyploid origin for wheat and oats suggested above, and consider that the typical features of many of the different species probably depend upon a balance of diverse factors (for which the extreme range of variation in some characters caused by environmental conditions perhaps provides some direct evidence), a scheme can be evolved for wheat which appears to fit the facts obtained from hybridisation studies equally well and to be in accord with the cytological evidence. This scheme would also fit most of the speltoid data. The genetic and cytological results are much more extensive in wheat than oats, which is the reason for this discussion on wheat, but there is evidence that the findings have almost equal application in both. Likewise an explanation which fits speltoid and fatuoid data must be considered in relation to the extensive facts obtained from hybridisation studies.

Winge (1924) represents the chromosome complement of the diploid *T. monococcum* as $\frac{1234567}{1234567}$ and *T. sativum* with its 42 chromosomes as a direct triplication of this: $\frac{1A\ 1B\ 1C\ \ 2A\ 2B\ 2C}{1A\ 1B\ 1C\ \ 2A\ 2B\ 2C}$ and so on to 7 inclusive. As the speltoid factors appear to be associated with only a single one of these seven groups of chromosomes, he puts aside the last six groups, and considers only the first, $\frac{1A\ 1B\ 1C}{1A\ 1B\ 1C}$. Then for the sake of brevity he drops the numbers and calls it merely $\frac{ABC}{ABC}$, which formula, for the purpose in hand, he takes to represent the genetic constitution of normal *T. sativum*. The B chromosomes are then assumed to carry factors for the speltoid type, and the C chromosomes to carry normal type factors. In normal wheat varieties the one A chromosome must usually pair only with the other A, the B with B, and the C with C, as otherwise, of course, the variety would not breed true to type. But if a faulty conjugation should occasionally take place between these chromosomes which though not identical are yet similar on account of their common origin, he points out that gametes ABB, and ACC may be formed. The mechanism producing these is visualised as $A + A \quad B + C \quad B + C$, and the gamete ABB is taken to be the one carrying an extra set of speltoid factors and lacking normal-type factors. Since such gametes are produced only rarely, such a one would practically always mate with a normal gamete, and the zygote $\frac{ABB}{ABC}$ would be formed. This formula would then represent a het. speltoid plant, which would be expected to produce hom. speltoid, het. speltoid, and normal progeny in the ratio of 1 : 2 : 1. The hom. speltoids would, of course, have the formula $\frac{ABB}{ABB}$. Gates (1923) had proposed a very similar scheme for some of the mutant types of *Oenothera* and found it capable of explaining many of the facts. It is a very attractive type of explanation if only as providing an hypothesis which is subject to test by microscopical means. Winge's findings of trivalent chromosome complexes in het. speltoid plants, and quadrivalents in hom. speltoids certainly lend themselves well to this general interpretation, and the genetic behaviour of A type speltoids is in good accord, since slight deviations from the 1 : 2 : 1 ratio would be expected where a whole chromosome difference instead of a single gene difference is involved. Winge's argument may be applied with equal validity to Type 1 fatuoids.

If the genetic constitutions of the different polyploid species within

the genera *Triticum* and *Avena* consist of different combinations of chromosomes from two or three primitive diploid species, as appears probable, then formulae similar to those of Winge's may be composed to represent the different species, which will explain many of the peculiar phenomena observed. In respect to the principal internode length and glume characters which distinguish different species of wheat, for instance, *T. dicoccum* might be represented by $\frac{B_1B_2}{B_1B_2}$, *T. spelta* by $\frac{AB_1B_2}{AB_1B_2}$, *T. vulgare* by $\frac{ABC}{ABC}$, *T. compactum* by $\frac{AC_1C_2}{AC_1C_2}$, *T. durum* by $\frac{AB}{AB}$, *T. polonicum* by $\frac{CB}{CB}$, etc.

Naturally the genetic constitution of the different wheat species is not as simple as these formulae may seem to imply, and the formulae themselves are to be considered only as tentative. But such a scheme seems capable of explaining in a general way many of the puzzling results obtained by Malinowski and others from hybridisation studies. Simple recombinations would account for many features, and cytological irregularities such as those here described in fatuoids would produce others. Darlington (1927) has mentioned some possible results of these.

Physical changes such as "deficiencies" and cross-overs between "semi-homologous" chromosomes could produce such complications as are now commonly explained by gene mutation. This scheme would explain, for instance, why the cross *T. vulgare* × *T. dicoccum* produces some types similar to *T. durum* and *T. spelta*, as well as the parental types, in the F_2 and later generations; why *T. polonicum* × *T. spelta* gives some *T. dicoccum* types, etc., etc.; and on the other hand why *T. spelta* × *T. dicoccum* gives only the two parental types. It would also account for the fairly frequent occurrence of speltoids from *T. vulgare*, since minor irregularities in the reduction-divisions are to be expected in hybrid polyploids, even though they have long been established as species. Further, it would account for the fact that while *T. compactum* types are segregated in small numbers by some strains of speltoids, yet speltoids rarely if ever arise from *T. compactum*. The occurrence of *T. compactum* forms in limited and irregular numbers from certain crosses of *vulgare* types and the reverse phenomena would be easily explicable on this basis. At the same time it would not interfere with the explanation of the clearly Mendelian ratios obtained from crosses in which simple gene differences are involved. The necessity for imagining such inherently improbable mechanisms as "labile genes," for postulating

unlikely cytological behaviour or reduplication series, or whole series of linked lethal or inhibitory factors, as various authors have done, is eliminated. Hypotheses made on this basis from genetic data can be tested reasonably adequately and easily by existing cytological methods, and those based on cytological observations can be tested genetically. Cytological material can be collected from wheat or oat plants without seriously affecting the yield of seeds, except in the case of some dwarf types, and a closely correlated study can be made.

Returning now to features immediately concerned in the production of fatuoids and speltoids, it may be reiterated that the same group of linked characters which constitute the essential specific differences between *A. sativa* and *A. fatua* also constitute the differences between normal *A. sativa* and fatuoids.

Following the device outlined for different wheat species we may then compose formulae for *A. sativa*, *A. fatua*, and for the different types of fatuoids. These formulae are, of course, concerned only with the differential features of these forms. In matters of colour, vegetative characters, growth habit, etc., either of these species exhibits parallel ranges of variation. The fatuoids, as previously mentioned, are similar to the variety in which they occur in all respects excepting those comprising the fatuoid complex. They therefore cover the same range of variation as *A. sativa* in the non-specific characters.

Using S to represent chromosomes carrying factors for the completely awnless *sativa* type, and F for those with *fatua* or fatuoid factors, with indices to indicate minor differences only, we may then for the present purpose represent the ordinary almost awnless type of *A. sativa* as $\frac{S_1 S_2 F}{S_1 S_2 F}$, if we assume that only two species are involved in its ancestry, or by $\frac{XSF}{XSF}$, if we assume three ancestral species. It makes no essential difference for the present which assumption is adopted, but different degrees of dominance must be postulated in the alternative formulae and the possibilities of reversion are limited at different ends of the scale. If we accept the latter formula $\frac{XSF}{XSF}$ to represent *A. sativa*, then Type 1 het. fatuoids would be $\frac{XSF}{XFF}$, and the hom. fatuoids from them $\frac{XFF}{XFF}$. If this formula is the correct one, then it follows that hom. fatuoids of this type cannot by internal rearrangement give rise to het. fatuoids or normal *A. sativa*. On the other hand, if the $\frac{S_1 S_2 F}{S_1 S_2 F}$ formula be adopted for *A. sativa*, then Type 1 hom. fatuoids would be

$\frac{SFF}{SFF}$ and in this case they could give rise to het. fatuoids and normal types. Whether this ever occurs or not remains to be proved; when proved it may settle several other questions besides indicating which of these formulae is the more correct. *A. fatua*, being similar to Type 1 hom. fatuoids in these respects, may be represented by $\frac{SF_1F_2}{SF_1F_2}$ or $\frac{XF_1F_2}{XF_1F_2}$. Het. fatuoids with 41 chromosomes would be $\frac{S_1S_2F}{S_1oF}$ or $\frac{S_1S_2F}{oS_2F}$ on the first alternative or $\frac{XSF}{XoF}$ on the second, and the sterile dwarf hom. fatuoids from them would be $\frac{SF}{SF}$ or $\frac{XF}{XF}$ respectively. Het. fatuoids with 43 chromosomes would be $\frac{S_1S_2FF}{S_1S_2F}$ on the first alternative or $\frac{XSFF}{XSF}$ on the second, and the sterile dwarf hom. fatuoids from them would be $\frac{S_1S_2FF}{S_1S_2FF}$ or $\frac{XSFF}{XSFF}$ respectively.

Direct evidence may never be available on the exact nature of the mechanism which causes the production of the SFF or XFF fatuoid gametes since they occur very rarely. Winge suggests that in wheat it is faulty pairing between the essentially similar but not identical semi-homologous chromosomes which causes ABB to go to one pole and ACC to the other. Gates (1923) suggests double non-disjunction for *Oenothera*, and has cytological evidence, unpublished, supporting this. It seems more probable that in oats a quadrivalent composed of $S + S + F + F$ analogous to that in *P. kewensis* is occasionally formed, and that upon the orientation of this in the heterotypic metaphase depends the alternative possibilities of $S + F$ going to each pole or $S + S$ to one and $F + F$ to the other. This would account for the fact that Type 1 fatuoids occur so much more frequently than the other types. Upon this hypothesis one would expect that fatuoids or speltoids which arise from the normal as chimaeras would usually be of the unbalanced types. The speltoid evidence supports this.

Until further genetic data are available, it does not seem advisable to attempt too close a codification of the different fatuoid types here described. From a 41 chromosome het. fatuoid, ratios of het. fatuoids and normals varying from 1 : 1 to 4 : 1 or even greater excesses of het. fatuoids, and very varying numbers of dwarf 40 chromosome hom. fatuoids, might be expected, the variation depending in the first place upon the number of times the split halves of the univalent fail to be incorporated in the daughter nuclei, in the second place upon the viability and functioning power of the different gametes, and finally upon the viability of the resultant zygotes. Environmental conditions must play

a very large part in determining the degree of variation incident upon these immediate causes. This will account for the very great deviations from Mendelian expectation which are found in any table of genetic results with speltoids or fatuoids, especially of the types other than 1 : 2 : 1. Such deviations are in sharp contrast to the results obtained in most cases where gene differences only are known to be involved.

In this connection I have been informed by Professor H. Kihara that he obtains different ratios from some of his 41-chromosome hybrid wheat plants when he grows them in different parts of Japan. The necessity for recording the number of plants eliminated during the growing season, and the advisability of attempting to determine if possible how far the elimination is differential with respect to the characters under observation must here be stressed also. With unbalanced chromosome types, the elimination is almost sure to be very heavy in certain cases. In het. fatuoid types 2 and 3 the mortality has here been shown to have been very severe in 1926. Many workers with speltoids have not recorded the number of seeds sown, and the number germinated, but Winge (1924) records that from 72 seeds of one C type speltoid he obtained 25 normals, 15 het. speltoids and 3 hom. speltoids,— a degree of elimination similar to that here recorded.

From a 43 chromosome form one might perhaps expect to obtain 42 and 43 chromosome plants in more or less equal proportions, and very few 44 chromosome plants. The limited genetic data from strain 26–13 did not conform at all well to this expectation in 1926. It will be instructive to note the progeny in 1927 of its daughter plants in which the individual chromosome number is known to be 43. It will also be interesting to compare ratios obtained from different sowings of seed from the same plants in both favourable and unfavourable environmental conditions.

Since C type speltoids can give rise to the B type, and the analogous change has also probably occurred in the het. fatuoid strain 24–22, one can draw no definite conclusions from individual chromosome counts until the particular plants cytologically studied have been propagated. This fact prevents discussion of the relationship existing between the Type 2 and Type 3 fatuoids here described until the progeny tests have been completed. Even then, if many of the plants are difficult to classify, a further test may have to be made.

Stanton, Coffman and Wiebe's (1926) discovery of "hom. fatuoids" which do not breed true to type is an extremely interesting one if the possibility of natural crossing can be ruled out. A cytological study of it would

then be very interesting as according to the conception here developed such a form might exist through having excess chromosomes bearing fatuoid factors. The 44 chromosome hom. fatuoids here described, if under any conditions they were fertile, would for instance almost certainly produce some het. fatuoid plants, through the chromosome irregularities observed in them,

The fact that all the dwarf fatuoid plants so far discovered have abnormal chromosome numbers may have considerable bearing on other cases of dwarfing. Of course, it is not suggested that all or even the majority of dwarfs known in the cereals are cytologically abnormal, but where they occur in peculiar and irregular ratios, or themselves give such progeny ratios, as many of those studied by Waldron (1924) and Goulden (1926) and others cited by these authors do, such a condition is certainly strongly suggested. Waldron made no cytological study, but Goulden found lagging chromosomes in most cells at the reduction-divisions, though the normal number were present. The findings of these authors again emphasise the undesirability of attempts to fit aberrant ratios to ordinary Mendelian schemes without first having determined whether the cytological conditions are normal or not.

It is interesting to find in fatuoids and speltoids, as well as in *Oenothera, Datura,* and to a lesser extent in *Drosophila* and other genera, that chromosome aberrations may produce segregation ratios which very closely simulate those expected from gene differences. These abnormal cases, of course, furnish considerable proof, if such be still needed, of the validity of the theory that the chromosomes form the basis for the transmission of Mendelian characters, and serve considerably to strengthen the pure Mendelian interpretation of normal inheritance.

Waldron states that with a single exception the dwarf characters reported in the self-fertilising cereals have become manifest only through crossing. While this statement cannot to-day be accepted at its full value, it does appear to be still essentially correct. He questions from this basis "whether the germplasm of the self-fertilised cereals may not have some sort of a protective or self-regulating device whereby such injurious lethal or semi-lethal mutations as dwarfs are accompanied by other protective mutations." It seems more probable that the crossing of different varieties or species introduces chromosome incompatibilities in the offspring and that many of these dwarfs may be chromosome mutations with either the normal number of chromosomes and incompatibilities, or with excesses or deficiencies.

For the same reason, it is possible that either natural or artificial

hybridisation in oats may have an indirect effect on the frequency with which fatuoids occur, though they certainly cannot be considered as segregation products of a cross in the ordinary sense of the term. Fatuoids have been found in some of the oldest and best established varieties of oats, as well as in the very newest productions of plant breeders, but they do seem to occur more frequently in the newer varieties. It is quite conceivable that the crossing of diverse normally self-fertilised lines might introduce disharmonies in the chromosome complement of the progeny which would cause the more frequent occurrence of chromosome aberrations of the types which produce fatuoids. This possibility may be found to link some of the views of Zade and Tschermak, who now ascribe the origin of fatuoids to delayed segregation following a cross, with those of Nilsson-Ehle and others who support the mutation theory of their origin.

The occurrence of fatuoids in cultivated oats is a fairly common source of annoyance to growers of high grade seed oats, and it was a request from Alberta farmers for further information about fatuoids which led to the initiation of the present study. The first result of importance to farmers was the demonstration that fatuoids have no direct connection with *A. fatua*, and that as they do not have the property of delayed germination, as the latter species has (Howes 1908, Garber and Quisenberry 1923) they do not constitute a serious weed menace. It would, however, be a matter of considerable importance if a variety of oats could be discovered or produced which would not give rise to fatuoids. In the course of some hybridisation experiments with *A. sativa gigantica*, some evidence, not yet available for publication, was obtained that this species is different with regard to awn factors from ordinary *A. sativa*, and the possibility arose that it might therefore differ in regard to the production of fatuoids. No fatuoids have ever been reported from this species, but, on the other hand, it has never been grown on a very large scale. It was, however, a matter of great interest to find that Professor Shegalov has a variety of *A. sativa* which gives results similar to those found in *A. sativa gigantica* hybrids, and that though this variety is very widely grown in Central Russia, fatuoids have never been found in it. Unfortunately this variety is not of high commercial value. While it is far too soon to express an opinion on the matter, the interesting possibility arises that *A. sativa gigantica* and these Russian oats either lack fatuoid factors entirely or have more factors for the normal *sativa* type than have ordinary varieties of *A. sativa*. If so, it may be possible by hybridisation with them to obtain good varieties of oats which will

not give rise to fatuoids. This breeding possibility is partly analogous to the situation in regard to black colour in oats, which Åkerman (1924) has put to practical application at Svalöf. The ordinary black oats of Sweden have a single factor for their black colour, and by mutation often produce white grains which spoil the appearance of the seed samples. By crossing these ordinary black oats with an inferior variety which was found to carry two factors for black, he has produced a valuable variety of black oats which very rarely produces white grains. It is possible that *T. compactum* occupies a position in regard to speltoids analogous to that of the Russian variety of oats which has never been known to produce fatuoids.

Summary.

In heterozygous fatuoids which give different segregation ratios, different distinctive cytological conditions have been found. The combined cytological and genetical evidence seems clearly to show that fatuoids arise from normal oats, neither by natural crossing between *A. sativa* and *A. fatua* nor by gene mutation, as previous authors have believed, but by chromosome aberration.

The ordinary practically awnless type of *A. sativa* is conceived as being the resultant of diverse factors present in its constitution on account of its polyploid and probable hybrid origin. It is the upsetting of this balance by chromosome aberration which produces fatuoids.

The commonest type of het. fatuoids of which many strains have been studied, segregates normals, het. fatuoids, and hom. fatuoids, all of approximately equal vigour, in a ratio of about 1 : 2 : 1. These segregates all have the normal chromosome number, viz. 42, but characteristic irregular arrangements are fairly frequently found in the hom. and het. fatuoids. Pollen-mother-cells of the normal segregates regularly have a chromosome complement at diakinesis and the heterotypic metaphase of 21ɪɪ. The het. fatuoids, on the other hand, frequently have 19ɪɪ + 1ɪɪɪ + 1ɪ, and the hom. fatuoids 19ɪɪ + 1ɪᴠ. The hypothesis which best fits this genetic and cytological behaviour is that an occasional aberration (probably the formation of a quadrivalent) in the meiotic divisions of normal cultivated oats produces a gamete in which one particular chromosome bearing *fatua* or fatuoid factors is duplicated, and another bearing normal-type factors is absent. The union of this gamete with a normal one would produce a Type 1 het. fatuoid.

Two strains have been obtained of a second type. The single panicle found in 1924 from which the first originated gave 6 het. fatuoid, 7 normal,

and 1 dwarf sterile, hom. fatuoid progeny. The second strain of this type obtained in 1926 has given 57 het. fatuoids, 37 normals and 3 sterile, dwarf hom. fatuoids. One of the het. fatuoids from the first strain was found to have only 41 chromosomes which were regularly arranged as 20ɪɪ + 1ɪ in the heterotypic divisions. This and a sister het. fatuoid gave rise to a third type in which het. fatuoids and normals occur in a ratio of about 4 : 1 plus a few sterile dwarf hom. fatuoids. These latter have only 40 chromosomes, and their meiotic divisions are completely irregular. Five of the het. fatuoids from this most recent generation have all been found to have 41 chromosomes arranged with only a very few exceptions similarly to those of the parent as 20ɪɪ + 1ɪ.

A fourth type has recently been obtained in which the het. fatuoids have 43 chromosomes which are arranged either as 20ɪɪ + 1ɪɪɪ or 21ɪɪ + 1ɪ. The hom. fatuoid segregates are all dwarf and sterile and have 44 chromosomes arranged either as 22ɪɪ or 20ɪɪ + 1ɪᴠ. The divisions are not very irregular but the contents of the pollen grains degenerate during the later stages of their development. Difficulties of classification make it impossible to determine the exact genetic behaviour of this strain until progeny tests have been made, but the present limited results approach a 1 : 2 : 1 ratio.

Biometrical studies show few if any significant differences in the vigour of the different segregates from the first type, which all have the same chromosome number, but where there are chromosome excesses or deficiencies the difference in vigour is marked.

The close analogy between fatuoids and speltoids is demonstrated, and an attempt is made to correlate the data from these forms with that from hybridisation studies in wheat and oats. Some suggestions are made concerning the genetic constitution of various species of wheat and oats.

The segregation ratios resulting from whole chromosome differences are shown to simulate those from gene differences, but to be more variable. The importance of correlating cytological studies with genetics in cases where dwarf types appear in irregular or unexpected ratios is evident from the aberrant cytological conditions here found in dwarf oats.

There appears to be a possibility of producing commercially valuable varieties of oats which will not give rise to fatuoids.

Chromosome counts are given for twelve species of *Avena*.

gestion of Professor J. R. Fryer, and continued there until August 1925, financial assistance being received from the Research Council of Canada during the last four months of that period. With the aid of an 1851 Exhibition Science Research Scholarship it has since been carried on in the Department of Botany, King's College, University of London, under the general direction of Professor R. R. Gates. Facilities for growing plants and preparing cytological material and help in seeding and harvesting have very kindly been provided at the John Innes Horticultural Institution, Merton, Surrey. The statistical work has been carried out in the Biometrical Laboratory, University College. I desire to express my thanks to those who have kindly provided the various facilities, to those who have supplied seeds of fatuoids and other oat varieties, and to Professor Gates for his continuous interest and assistance.

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EXPLANATION OF PLATES XIX—XXI.

PLATE XIX.

Strain 26–24, figs. 1, 2 and 3: homozygous fatuoid, heterozygous fatuoid and normal type segregates respectively, all having 42 chromosomes; from Type 1 heterozygous fatuoid F_1 plant of homozygous fatuoid Sir Douglas Haig × normal Sir Douglas Haig. Enlarged figures: lower—a spikelet with outer glumes removed; upper—a similar spikelet with florets separated.

Strain 25–98, figs. 4, 5 and 6: sterile dwarf homozygous fatuoid, heterozygous fatuoid and normal type segregates with 40, 41 and 42 chromosomes respectively; from Type 2 heterozygous fatuoid Victory plant 25–98, having 41 chromosomes.

PLATE XIX

PLATE XX

Scale

Panicles Grains

inches
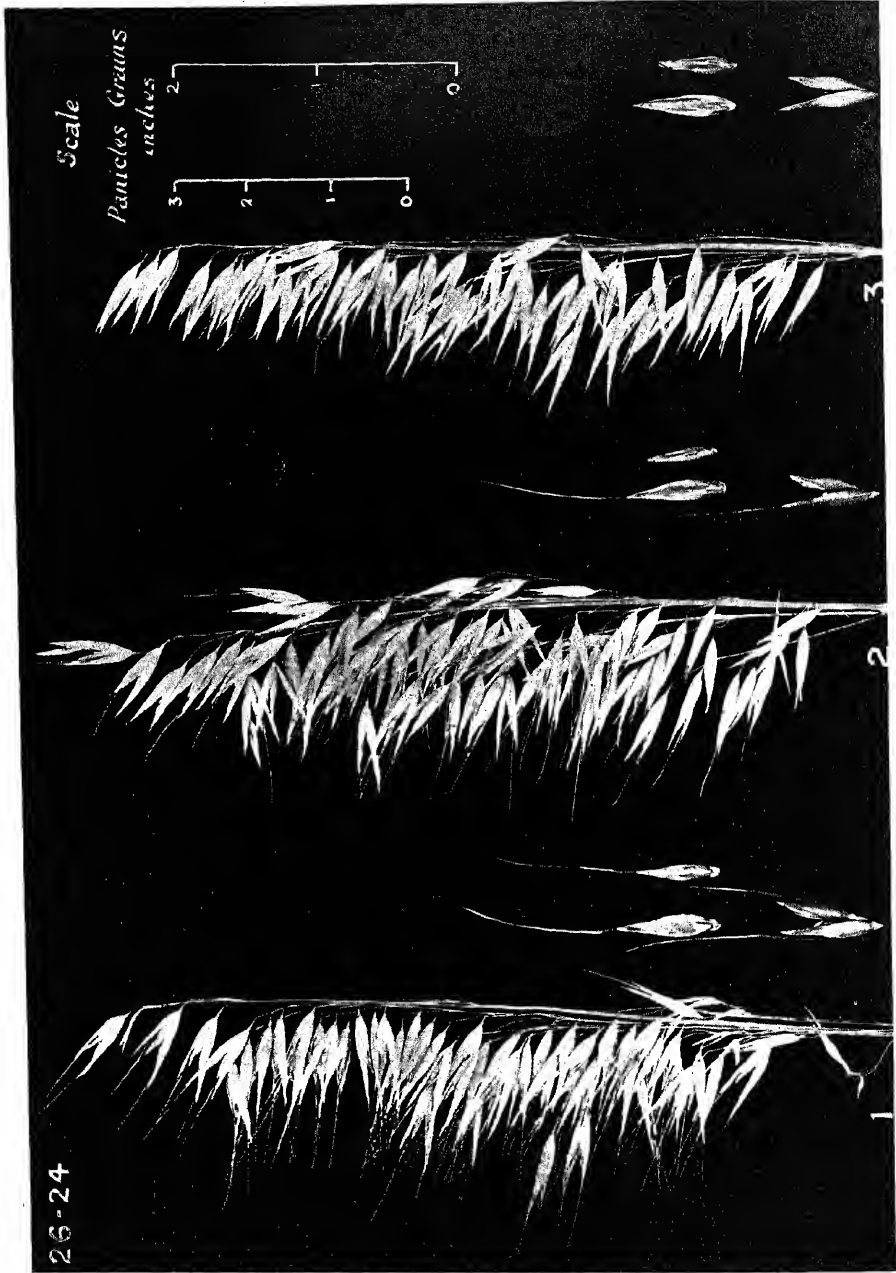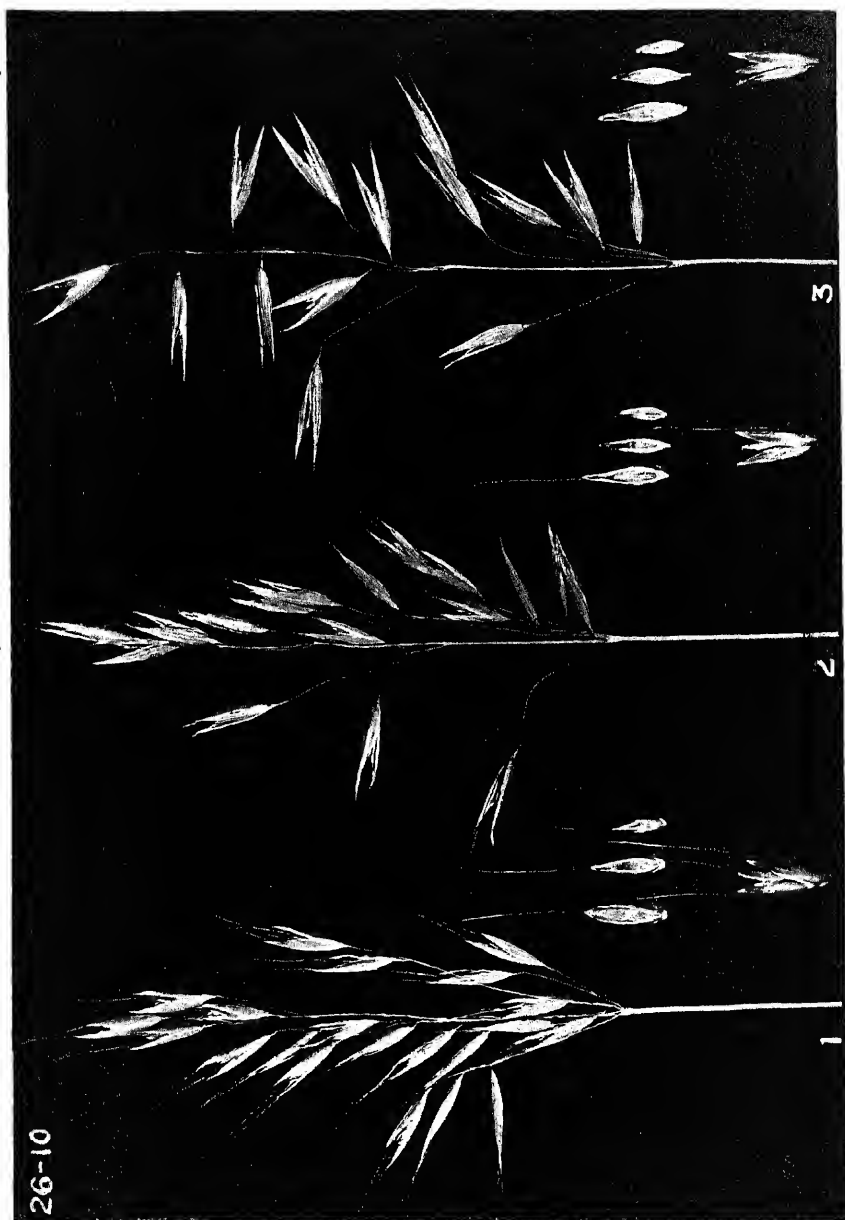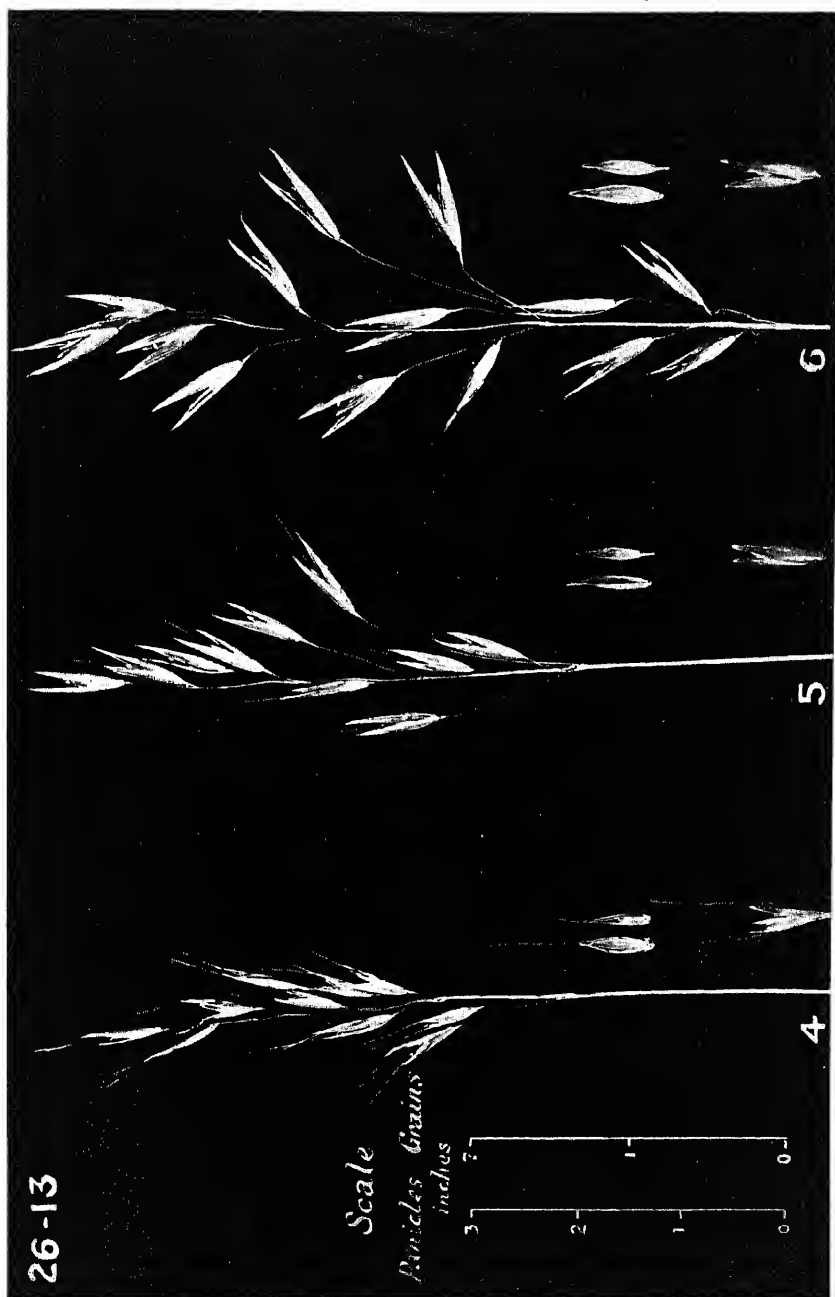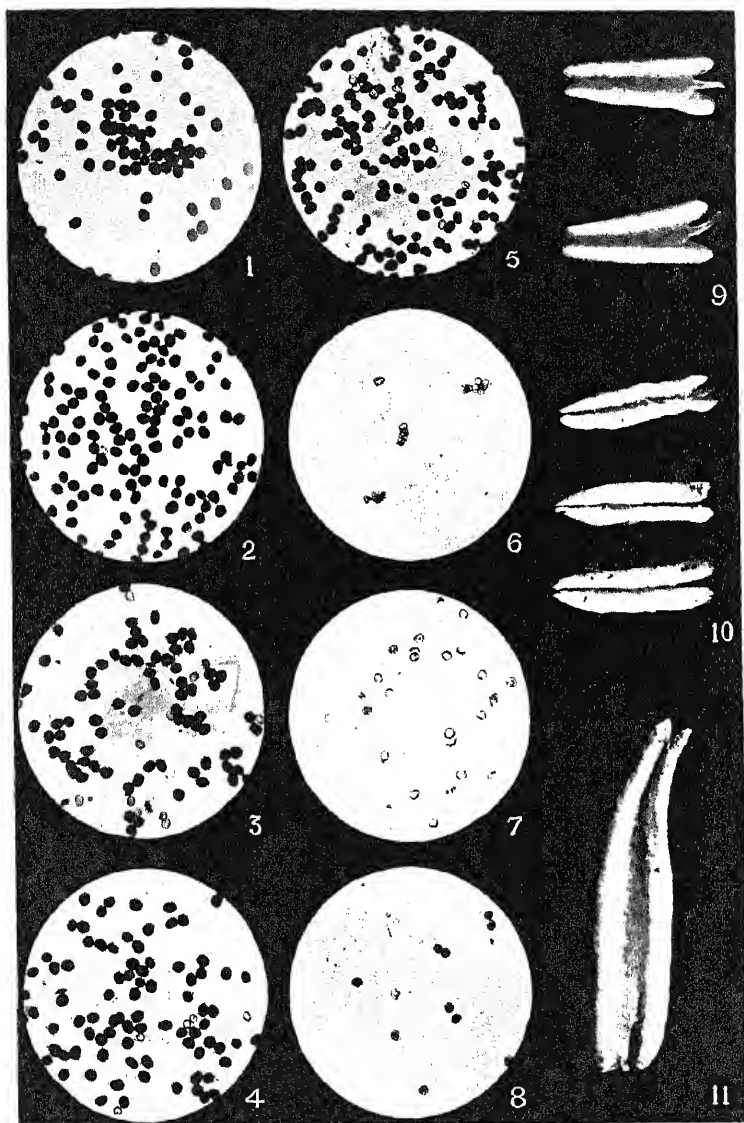
THE INHERITANCE OF HORNS IN CATTLE.
SOME FURTHER DATA.

By A. D. BUCHANAN SMITH.

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(With One Plate and Four Text-figures.)

WHEN the writer first heard of certain exceptions to the well-estab-lished rule of simple dominance of the polled condition in cattle, he paid little attention to them. Further records of such exceptions, however, were encountered, and it was then felt that the subject merited fuller consideration. The following report concerning such exceptions came from two main sources: from Northern Rhodesia, where the crosses concerned were Aberdeen-Angus bulls and native cows, and from England, where crosses involving the Park Cattle, between their two types and various other breeds, appeared to give anomalous results.

A. RHODESIAN NATIVE CATTLE.

For precise information regarding the case in Northern Rhodesia, the writer is indebted to Mr R. A. S. Macdonald, M.R.C.V.S., of the Veterinary Experimental Station, Chilanga. His facts relate mainly to two cases, and are as follows:

1. In a herd of Angoni and Mashukulumbwe cows, the majority of which were pure native bred (though a few showed slight traces of Hereford or Shorthorn blood, but none had any Aberdeen-Angus blood), a pure bred registered Aberdeen-Angus polled bull was placed (see photograph). The cows were all horned, and in colour were red, red-and-white or dun. There were practically no blacks. Fifty-one of their progeny so produced were traced as under:

> 27 heifers all completely polled.
> 9 bulls all completely polled.
> 15 bulls mostly with distinct horns or buds.

All were completely black with the exception of a few white faces. This was the case in 120 such calves.

2. In another herd, a pure bred registered Aberdeen-Angus bull (polled) was mated to native cows, all of which possessed the typical

heavy horn. In this case, all the progeny were completely black, practically all the heifers polled, and practically all the males with buds, short drooping horns, or ordinary short horns.

It was found difficult to obtain more detailed information as regards exact numbers, and rather than obtain figures which could not be guaranteed to be correct, it was considered advisable merely to give the bare outline of facts. The following conclusion concerning these two herds, which are typical of several others, may, therefore, be considered indubitable and has been confirmed by correspondence:

That a registered Aberdeen-Angus bull (polled) when mated to the native cows of Northern Rhodesia (horned) produced calves, all the heifers being polled and without scurs, and the majority of the bull calves possessing horns of various shapes, a few being only scurred, and a very few being polled.

As the cattle of Northern Rhodesia are being rapidly "graded" up, it is unlikely that the native breed will retain their purity much longer. And since it seemed improbable that more exact facts were likely to be obtained, or that the reciprocal cross was likely to be made, it is considered advisable to record these facts as they stand.

The conclusion, therefore, is that in matings with the indigenous cattle of Africa, horns are inherited as a sex-limited character; that is to say that the factors for their expression are either dominant in the male and recessive in the female, or otherwise governed by sex. Since, in the ordinary domesticated breeds of cattle, the polled condition behaves as a simple dominant to the horned, and since there was some blood of domesticated breeds in certain of the native cows used in this cross, it would appear logical to presume that either the sex-limited transmission of horns, or the simple dominance of the polled condition, is the rule in cattle, and the one condition may be altered to the other by the action of one or more pairs of modifying factors.

A point worth noting in this connection is that although this cross reveals a sex difference in respect of horns, castration of the males does not appear to modify the horns to any great extent, as is the case in other animals whose horns are modified or conditioned by sex, *e.g.* sheep (Marshall and Hammond, 1914), though Arkell (1912) shows this to be not so marked in the case of Merinos.

B. Park Cattle.

The report concerning the other exception to the rule of simple dominance of the polled condition in cattle, came from the Wild White

Park Cattle breeders in their operations towards the formation of the domesticated breed of Park Cattle. Since 1923, the writer has been in touch with several breeders in an effort to obtain data regarding the inheritance of coat colour in crosses of Park Cattle and other breeds. At first, no record was taken regarding the inheritance of horns, as the figures appeared merely to be abnormal ratios. But, since practically all the breeders with whom contact was made had obtained quite unexpected ratios of horned and polled calves, resulting from certain crosses, it was considered advisable to obtain some further reliable data. The data which follow were obtained from Sir Claud Alexander, Bart., of Faygate, Sussex, and Mr A. H. L. Bohrmann, F.Z.S., of Ide Hill, Kent, both of whom are leading breeders of Park Cattle, and recognised authorities on the breed.

Before tabulating their experiences, it must be stated that there are, at the very least, two distinct types of Wild White Park Cattle which form the foundation stock for these experiments. These are called the horned and the polled. The horned were formerly the more common in the north and west of Great Britain, and are now best represented by the Chillingham Herd in Northumberland belonging to the Earl of Tankerville, and the Cadzow[1] herd in Scotland, the property of the Duke of Hamilton. The former herd has had very little, if any, addition of foreign blood within the past two hundred years. The latter is not quite so pure, yet has a definite type, though some West Highland blood has been introduced into it as well as some Chillingham. The

[1] Of this herd, Mr Brown, Chamberlain to the Duke of Hamilton, reported about the year 1835, that, "The cows seldom have horns" (Storer, 1879, p. 339). This was corroborated by Sir John Orde of Kilmory, Youatt, Macgillivray (in his prize essay), and Mr Chandos-Pole-Gell. The latter in October 1874 found the herd entirely horned. Sir John Orde suggest sthat the herd acquired horns after the introduction of a Highland bull (Storer, pp. 341-345). (See also Auld, 1888, pp. 507-508.) The earliest known reference to this herd being polled is in 1809 when a local poet, Robert Burns of Hamilton, in the note to his poem, "Cadzow Castle," dedicated to Sir Walter Scott, wrote that the bulls then in use were mostly "humble" (i.e. polled) and that some of the cows had "fine moony horns." Sir Walter Scott himself in his early poem, "Cadzow Castle," written a year or two previously, but dealing with events of the year 1569, describes the death of a wild bull "with black hoof and horn," despite the fact that he was well acquainted with polled cattle, and a friend of Mr Hugh Watson, one of the founders of Aberdeen-Angus cattle. As the original wild Caledonian cattle were undoubtedly horned, it would appear as though the Cadzow herd became polled either by a mutation or the infusion of polled blood, possibly after the Cromwellian period, when, as Mr Brown says, it was "nearly extirpated." It is suggestive that, according to Burns, in 1809 the bulls were polled and cows horned, whereas on the evidence of later authorities, e.g. Mr Brown, the females were more frequently polled. This might indicate that polled blood was introduced through the bulls about the beginning of the century, just as the horned blood was several decades later.

Fig. 1. Horned Park bull from the Chartley Herd, "Faygate Brace." Though mated to many polled cows, this bull left only one polled calf, and that a twin to a horned calf. (By courtesy of Sir Claud Alexander, Bart.)

(Figs. 1–4 by courtesy of Mr R. A. S. Macdonald, M.R.C.V.S.)

Fig. 2. "Faygate Garter," a typical horned Park cow by a horned Park bull, and out of a polled cow from a pure polled herd. (Photograph by the owner, Mr A. H. L. Bohrmann.)
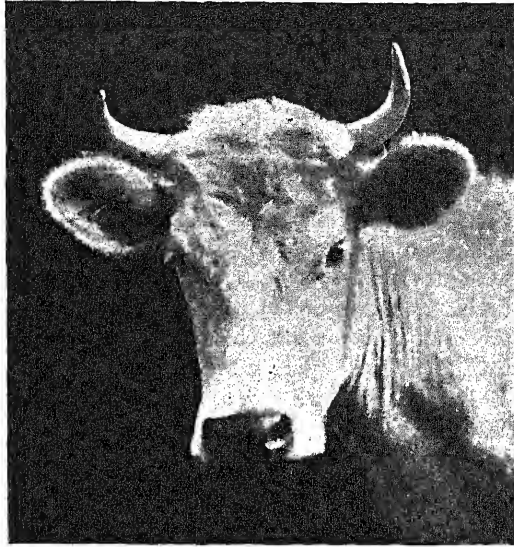
Fig. 3. Horned heifer, out of a horned Shorthorn-Ayrshire cow by a polled Park bull. This bull, when mated to a pedigree Shorthorn cow, produced a polled calf. (Photograph by Mr A. H. L. Bohrmann.)
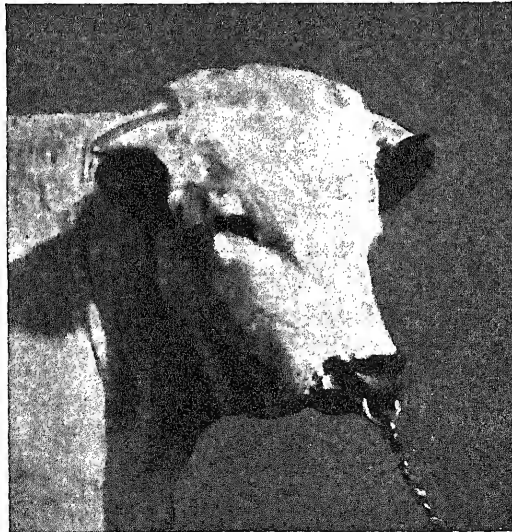
Fig. 4. Horned bull (14 months old) by a horned Park bull (white with black points) and out of an Aberdeen-Angus cow (polled, black). (Photograph by Mr J. Cole.)

Chartley herd, now belonging to the Duke of Bedford, is also horned. It contains some Longhorn blood (introduced after removal from Chartley to Woburn).

The polled Wild Park Cattle were located chiefly in the middle and eastern counties of England. The Somerford herd, one of the oldest, belonging to Sir Walter Shakerley, Bart., dispersed in 1925, probably occasionally threw horned calves. The Woodbastwick herd, belonging to John Cator, Esq., has some Shorthorn blood in it. For many years the latter herd provided the former with bulls.

There are now seven horned and ten polled herds, and two containing both kinds. The two sections of the herd book are kept quite distinct, and breeders who keep both horned and polled Park Cattle, have to register them as two separate herds. Entry is, however, allowed from inter-herd breeding, as also is admixture of blood from other breeds, provided the progeny are of the required type. It must therefore be remembered that many of the polled animals are heterozygous for that condition.

Sir Claud Alexander states that horned individuals crop up occasionally in polled herds. He further states that his herd was founded (1908) on cows culled from the original polled herds, mated to pure Cadzow and Chartley bulls (horned). The herd was occasionally replenished with cows from the polled herds, but of the calves produced by this cross over a considerable period of years, only one was polled, and that a twin, the sire being the horned pure old Chartley bull, "Faygate Brace."

Mr Dawkins at Wilcote bred Park Cattle for many years, mostly founded on white Pembrokes with crosses of other horned and polled Park Cattle. He crossed horned Park Cattle with polled Park Cattle, and also, experimentally, horned Park bulls with polled cows of various other breeding (including Aberdeen-Angus). From such horned-polled crosses he obtained 21 calves[1] of which 19 were horned and two polled (one bull calf and one heifer calf).

In the Lyme herd (horned) which belonged to Sir Piers Leigh, when a polled Gisburne bull from the Gisburne herd (now extinct) was mated to a horned cow, the progeny was fully horned, but the horns grew downwards instead of outwards. Whether there were any polled calves from this cross does not appear to be known. Of precise matings it is difficult to obtain data, as reliable figures as regards sex, etc., do not appear to have been kept.

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[1] The experimental crossbreds were all, later, culled from the herd.

Mr A. H. L. Bohrmann[1] has supplied the following: An Irish black Moolie cow (polled) calved to a Friesian-Park × Shorthorn bull (horned) and produced a horned heifer calf. A horned bull of various of the best Park strains and with one British Friesian grandparent, mated to an Aberdeen-Angus (polled) cow, has produced a horned bull calf. These together with other results are given in Table I.

TABLE I.

Summary of Crosses of Park Cattle, etc.

Herd	Sire and Dam	Offspring	Remarks
Faygate	Park H* (Cadzow) × Park P†	All H	From 1908 to about 1918, 30 calves
	Park H (Chartley) × Park P	All H save 1 (a twin)	
Wilcote	Park H × Park P	⎱ 19 H (8 ♂, 9 ♀)	
	Park H × various P including Aberdeen-Angus ⎰	2 P (1 ♂, 1 ♀)	
Lyme	Park P (Gisburne) × Park H	All H	Perhaps one or two P
Ide	Friesian/Park-Shorthorn H × Irish Moolie P	H ♀	
	Park (some Friesian) H × Aberdeen-Angus P	H ♂	
	Park H (Chartley) × Park P	H ♀ (a)	
	Red Poll-Park P × (a) H	P ♀	
	Park P × (a) H	P ♂	With scurs
	? H × (a) H	H ♂	
	Park P × Shorthorn-Ayrshire H	H ♀	Same bull mated to Shorthorns produced only polled

* H = Horned.   † P = Polled.

The proper interpretation of these cases really rests on the fact that an abnormally small number of polled calves have been produced by a mating of horned × polled, even when the polled parent was registered in a polled herd, or of a pure polled breed. Nevertheless, it must be remembered that normally these uneven ratios do not occur often, even in a large population, and that therefore, since the population from which they are culled is extremely small, their significance is the greater. No reliable data have been obtained of any cases where horned × horned have given polled offspring. Only the very general conclusion can be drawn that the Wild White Park Cattle appear to carry certain factors which modify the normal mode of inheritance of horns.

DISCUSSION.

An experience similar to these was recorded by Wentworth (1911) in the case of the famous White Shorthorn × Galloway cattle breeding

[1] Mr Bohrmann has bred a considerable number of crossbred cattle for experimental purposes only (not to augment his herd).

experiment at Iowa State College. With two exceptions, the 70 F_1 animals were all blue grey and polled. A polled F_1 bull was mated back to three Galloway cows (polled). By one, he sired only polled offspring, and all the calves this cow produced by horned bulls were also polled. By another, he produced a horned bull calf, though this cow had six polled offspring afterwards. By the third, he produced a horned bull and a horned heifer calf at different births, as well as four polled calves. It is probable that these last two cows were heterozygous for the polled condition, and that it was a chance that two such cows should have been used. But the fact that the horns of one of these calves resembled not a bit the horns of any pure breed of cattle, may lend some doubt to this statement.

Mossam Boyd (1906) mated a polled *mutant* Hereford bull to horned Hereford cows, and obtained the curious proportion of 22 polled to 6 horned calves. He also records (1908) data drawn from crosses of Bison bulls on 14 polled cows which seem to show that in this cross the inheritance of horns is the same as in domestic breeds of cattle, with the polled condition a simple dominant.

Gowen (1918, 1927) states that when the factors for the polled condition are in the simplex state, "they tend to produce the horned condition more frequently in the males than they do in the females." He gives an example of a black bull with short horns produced by a mating of an Aberdeen-Angus bull (polled) on an Ayrshire (horned) cow. Spillman (1905) had already suggested this but without adducing any evidence. Watson (1921) also found the polled condition to be occasionally incompletely dominant in the male.

There is a further point to which reference may be made, namely, the condition as regards horns of early forms of cattle. The majority of authorities recognise that it was a polled animal that was the forerunner of the present-day cattle. It has, however, been pointed out by many writers that these polled types were succeeded by species in which the males were horned and the females polled. Prof. J. Cossar Ewart (1909) states: "Several species of cattle with horned males, but hornless females, occur in the lower Pliocene deposits. In *Leptobos Falconeri* of India, *L. Etruscus* and *L. Latus* of Italy, France and Spain, we have early Pliocene species in which horns were probably always absent in the females. In the deer group, the females as a rule have continued hornless, but during the later Pliocene period, female as well as male oxen acquired horns, and the horns seem to have been retained by both the males and the females until cattle were domesticated during the

neolithic age." This view is substantiated by Boyd, Dawkins, Auld, Lydekker, Rutimeyer, Arenander and others.

This therefore inclines one to the conclusion that the simple dominance of the polled condition is derived from an earlier sex-limited condition which is still to be found in certain types of cattle, especially those in which there has been little or no admixture of the recognised modern domesticated breeds.

In the genetic acquisition of horns, cattle seem to illustrate a general evolutionary principle. The mutation from the polled to the horned condition was first exhibited by the male, and only much later in the history of the species by the female.

As no accurate data were obtained concerning scurs or slugs, no study of these has been made, though the results of Watson (1921), Cole (1924) and Landauer (1925) would seem to show that their expression is largely sex-controlled.

CONCLUSION.

There are numerous records which confirm as a general rule the simple dominance of the polled condition in cattle, and it is unnecessary to quote them in detail as the fact has been well established by the common practice of cattle breeders. The purpose of this paper is to show that there appear to be factors which modify the normal mode of inheritance. The writer in no way claims that the data presented in this paper deny the established rule, but rather suggests that in certain crosses there are additional complications, and that these complications appear to arise when blood strains other than those of the commoner domesticated breeds are present. This appears to be a perfectly logical evolution of the study of the inheritance of horns, and may be compared to the progress of the studies of many other heritable characters in both plants and animals.

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See also LLOYD-JONES, ORREN and EVVARD, J. M. (1916). "Inheritance of Colour and Horns in Blue-Grey Cattle." *Agricultural Experiment Station, Iowa State College, Research Bulletin,* No. 30.

EXPLANATION OF PLATE XXII.

Fig. 1. "Rhodesdale Magistrate," a pure bred Aberdeen-Angus bull used in case 1.

Fig. 2. A typical Mashukulumbwe cow showing the horns, with a 6-months old grade calf at foot.

Fig. 3. The male progeny of a cross of such a bull as shown in Fig. 1, and the cow as shown in Fig. 2, and others like her. The bullocks are two years old. Note the various types of horns.

Fig. 4. The female progeny of the same cross, all completely polled. They are two years old.
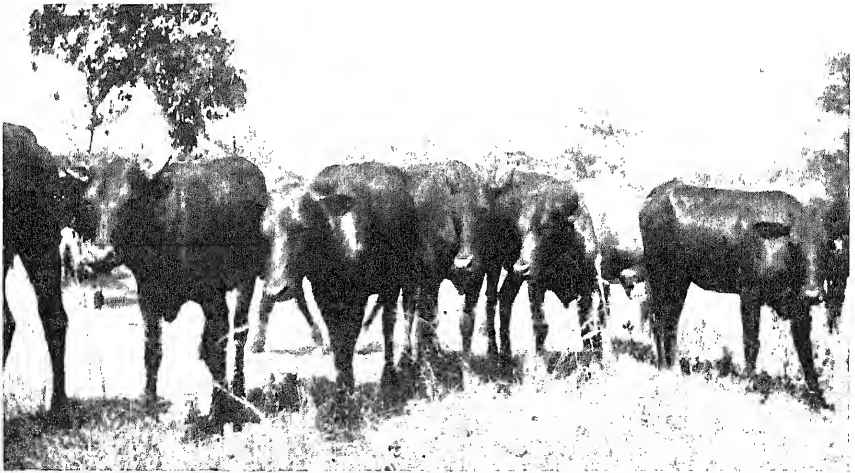
Fig 1

Fig. 2.

PLATE XXII
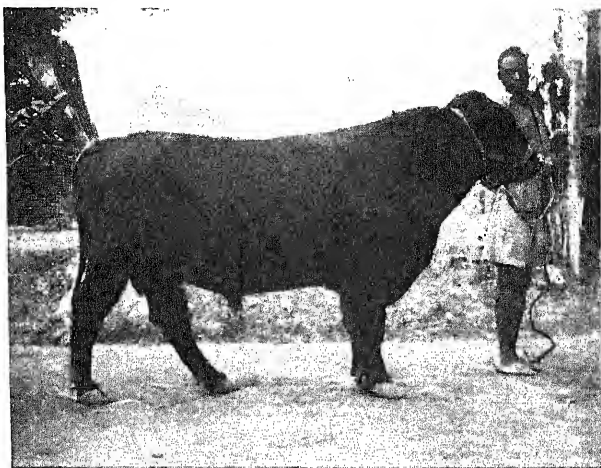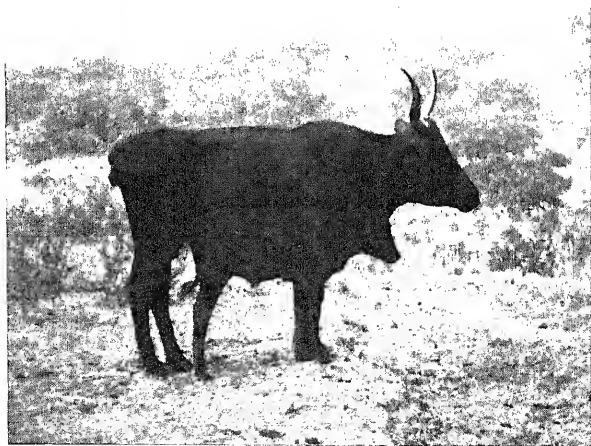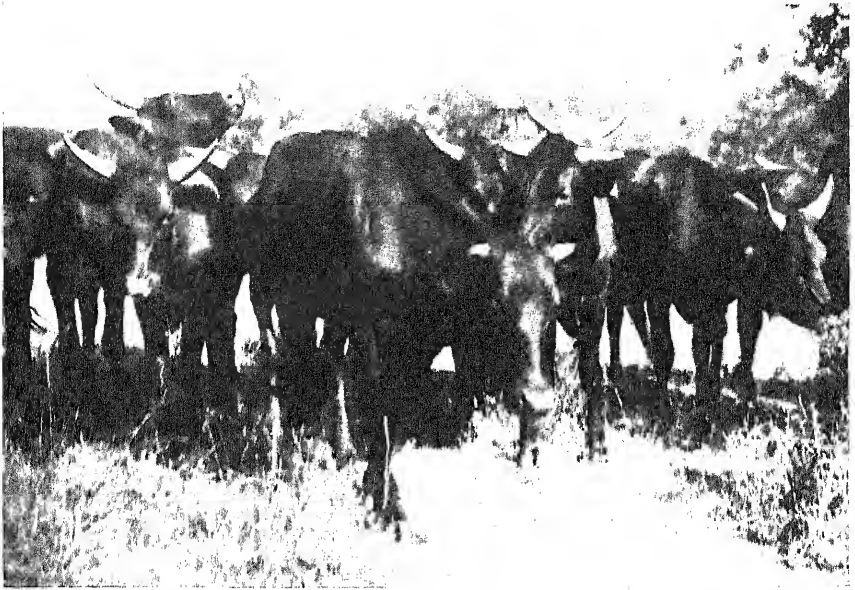
Fig. 3.

Fig. 4.

GENETIC AND CYTOLOGICAL STUDIES
IN WHEAT. III.

By A. E. WATKINS, M.A.

(Plant Breeding Institute, School of Agriculture, Cambridge.)

(With Two Plates and Eight Text-figures.)

CONTENTS.

INTRODUCTION.

In the two previous papers of this series (5, 6) I have given the main outlines of the cytology of crosses between the 14 (haploid) chromosome species *T. turgidum* and the 21 chromosome species *T. vulgare*. It was there shown that the egg-cell and pollen grain nuclei of the F_1 may contain any number of chromosomes from 14 to 21, the intermediate numbers being much the most frequent; that all the egg-cells are functional, but only a limited proportion of the pollen grains. In this paper it is proposed to give a preliminary account of the genetics of the cross, together with a more detailed account of the genetic aspects of pollen sterility.

After describing the wheat species *turgidum* and *vulgare* I have dealt with the general nature of the F_2 obtained when they are crossed and with the types that are extracted from this and later generations. This account is based on an F_2 from Rivet × Iron, of which about 800 plants were raised in 1921, on a complete F_3 raised the following year, and on various extracted types that have been carried on to F_6 or F_7; on other F_2's and F_3's from the same cross and from Rivet × Yeoman; and finally on the results of reciprocal back crosses made between the (Rivet × Iron) F_1, and the (Rivet × Yeoman) F_1, and the parent forms.

Experience of the association of type of plant with its chromosome number has been gained by counts made for some 50 or 60 plants, supplemented by the knowledge (5) that the progeny of plants with less than 35 chromosomes will finally settle down to the 28 chromosome condition, while similarly plants with more than 35 chromosomes will give 42 chromosome types; also by knowing that all plants obtained by crossing the F_1 to *turgidum* will contain from 28 to 35 chromosomes, and those from crosses between the F_1 and *vulgare* from 35 to 42 chromosomes.

In most crosses between distinct species, sterility in the F_1 and later generations makes an accurate genetic analysis impossible. In such crosses it is usual to assume that the difference between the species can be explained on the basis of Mendelian factors alone, and the irregular crossing results are considered as caused only by irregular chromosome behaviour and infertility. Much support can, no doubt, be found for this view; and to explain sterility as the elimination of all gametes or zygotes that diverge widely from the parental types certainly seems reasonable. But it is often difficult to avoid the feeling that the problem of species involves more than this; that the mechanism of segregation may not be quite the usual one, or that species characters may not be determined by factors in quite the same way that the characters that separate varieties are. The wheat cross we are considering here should enable us to gain some enlightenment on these points since all the F_1 egg-cells are fertile, so that by crossing back to the parents we can study the genetics of the egg-cells with certainty so long as we are able to distinguish the intermediate from the type form of any character. Further, the F_1 pollen is partially sterile, and we should be able to verify the current theory of sterility by comparing reciprocal crosses between the F_1 and the two parent varieties. In making this comparison, the plants have been classified into types—*turgidum* type, F_1 type, etc.—wherever this could easily be done. This classification is based largely on the study of certain characters that will be indicated; but an actual study of the mode of inheritance of these characters, and their representation by factors, will be considered in a later paper.

The following abbreviations have been used:

F_1 = *vulgare* × *turgidum* F_1, unless the contrary is stated.

$F_1 ♀ × V ♂ = F_1 ♀ × $ *vulgare* ♂.

$F_1 ♀ × T ♂ = F_1 ♀ × $ *turgidum* ♂.

$V ♀ × F_1 ♂ = $ *vulgare* ♀ $× F_1 ♂$.

$T ♀ × F_1 ♂ = $ *turgidum* ♀ $× F_1 ♂$.

The Wheat Species *turgidum* and *vulgare*.

(a) *Description of the species.*

Both species include a wide range of forms, and reference will be made chiefly to the forms used in this investigation and to certain characteristics of the species that have been made the subject of genetic study. A full account is given by Percival in his monograph (4). From each other the two species are sharply defined both in vegetative and in ear characters, as well as by their difference in chromosome number. With regard to their relation to other species in the genus, *vulgare* is well separated from the species belonging to the same group, but *turgidum* is not easily separated from the closely related *T. durum*.

As before, the varieties used were Rivet (*T. turgidum*), and Swedish Iron and Yeoman (both *T. vulgare*). In most of the work Swedish Iron was used as the *vulgare* parent; and as the results obtained when Yeoman was used were similar in most respects I shall not hereafter discriminate between the two cases unless there is need.

Ear characters. In both species the ears vary greatly in density, but very lax forms are probably only found in *vulgare* and on the whole it may be said that greater ear density is a characteristic of *turgidum*. The latter always has bearded ears, but in *vulgare* both beardless and bearded forms occur; beardless *turgidum* can readily be produced by crossing the two species. In both species the chaff may be rough or smooth and the glumes red or white in colour. In *turgidum* the glumes may be grey; in *vulgare* I have not seen this colour, but Percival describes several varieties with the glumes of various shades of grey. Rivet (Plate XXIII, fig. 1 *a*) has dense ears, with rough, grey chaff. Both the *vulgare* forms have smooth, white chaff, and are beardless; both are dense eared, but Swedish Iron is the denser (Plate XXIII, fig. 1 *c*).

The shape of the glume is very important; and glumes from the varieties under consideration are shown in Fig. 1 and in Plate XXIV, fig. 3. The Rivet glume type is never found in *T. vulgare*, nor the Swedish Iron type in *T. turgidum*. The prominent keel of the Rivet glume (see Fig. 1) is common to all forms of *turgidum*; and though some *vulgare* forms have a slight keel running to the base it is never well marked, and in most forms it is practically absent from the lower half of the glume. All forms of *vulgare* have the glume inflated (Fig. 1), while in *turgidum* it is compressed as in Rivet. In both species, however, the shape of the glume shows a fair amount of variation, probably more in *vulgare* than in *turgidum*, and I doubt whether there are any other

features of the shape that are both common to all forms of the one species and absent from all forms of the other; though there are sometimes other features that are found only in the one species—for example, the flat top of some of the glumes shown in Plate XXIV, fig. 1 is found in some forms of *vulgare* but never in *turgidum*. No doubt there are many other differences that might be mentioned, but these are chiefly
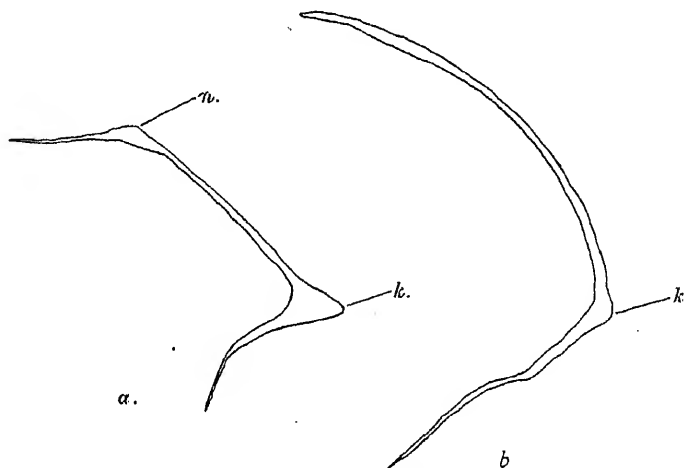

Fig. 1. Transverse sections through centre of glumes to show keel. *a, T. turgidum* var. Rivet; *b, T. vulgare* var. Swedish Iron. *k*=keel, *n*=secondary nerve. Magnification × 12.

of a trivial nature, and though the expert can readily detect the fact that differences exist, definition and description of the differences are difficult to make.

The Straw. In Iron, Yeoman, and nearly all varieties of *vulgare* the straw is straight; while in Rivet, and all other forms of *turgidum*, it is curved below the ear. In Iron, Yeoman, and nearly all *vulgare* wheats, the straw has thin walls, contains very little pith, and is therefore hollow throughout its length; but in a few forms of *vulgare* it is solid. In all forms of *turgidum* the straw is filled with pith (solid), or is solid at the top with a small lumen in the lower parts; Rivet is of the latter class. It should be mentioned, however, that crosses between *turgidum* and the closely related 28 chromosome species *T. durum,* which also has a solid straw, give forms with the straw less solid than that of either parent (1). As with all other *turgidum* forms, the straw of Rivet is slender, especially just below the ear, and very tough and pliable; it is circular in cross-section, but the surface is ribbed due to bands of fibres that run longi-

tudinally down it (Fig. 2). In *vulgare* it has a greater diameter, is neither so tough nor so pliable, and is more elliptical in cross-section, with a smoother surface owing to the absence of the ridges of fibres.

Leaf characters. According to Percival(4) one of the most important characters to be considered in assigning the various wheat forms to their appropriate species is the arrangement of the hairs on the young leaf.
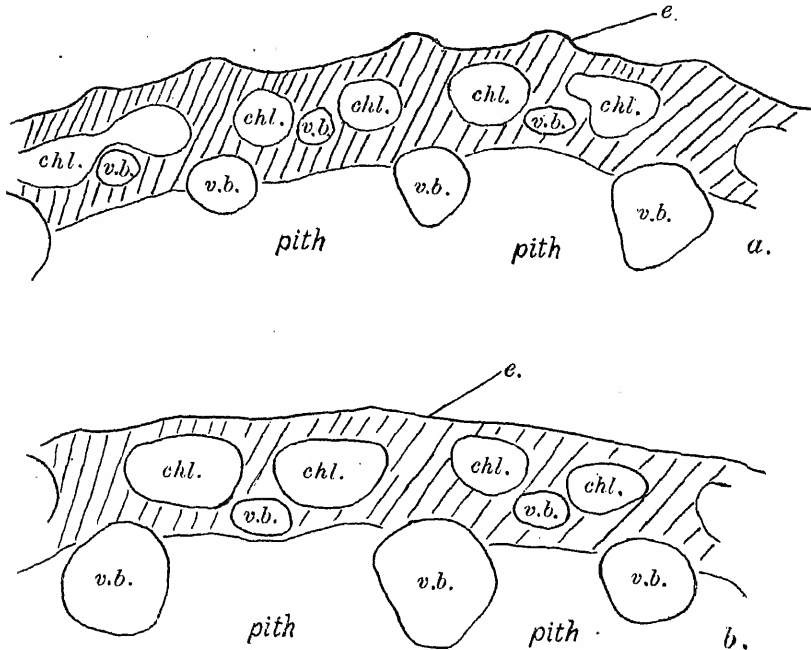

Fig. 2. Part of transverse sections of straws. *a*, *T. turgidum* var. Rivet; *b*, *T. vulgare* var. Swedish Iron. *e* = epidermis, *chl.* = degenerated chlorophyllous tissue, *v.b.* = vascular bundle. The shaded portion consists of fibres. In Rivet, ridges of fibres run longitudinally down the straw opposite the vascular bundles.

I have accordingly paid some attention to this character, but here it will be enough to mention that *turgidum* and *vulgare* show a constant difference in this respect: a fuller description of the character and the way in which it is inherited will be given in a later paper.

In shape, the older leaves of Rivet are narrower, longer, and more pointed than those of Iron; they are also of a more greyish green, as distinct from a bluish green colour, but this difference can only be seen easily when the two varieties are grown side by side in bulk. These differences are inherited in the manner of specific differences, as will be shown later, but I am uncertain to what extent the two species can be

differentiated in this way, and certainly narrow-leaved forms of *vulgare* are common.

(b) *The F_2 and the extracted types.*

The F_2 from the species cross is extremely variable, containing new forms unlike either parent or any other known wheat variety; and, judged by ability to produce grain, shows every gradation from complete fertility to complete sterility. From the behaviour of the chromosomes it should be possible to view the genetic results as falling into two classes. On the one hand the 14 *turgidum* chromosomes pair with 14 from *vulgare* and, so far as can be told, segregate normally; here therefore normal Mendelian inheritance should result, perhaps with complications if any of the factors have an influence on sterility. In the case of the other 7 *vulgare* chromosomes, however, we have a very different result. These are distributed at random to the gametes, and the latter, by their union, produce F_2 zygotes with any number of chromosomes from 28 to 42, though not in every possible combination (5, 6). The genetic effects we have to consider are two: first, the effects produced on 28-chromosome plants by adding random selections of from 1 up to 7 of a single set of the extra *vulgare* chromosomes, producing thereby plants with from 28 to 35 chromosomes; secondly, by increasing the chromosome number of a plant from 35 to 42 we merely change the extra chromosomes from the univalent to the bivalent condition—we do not add anything new. The effect of this addition of chromosomes to a 28-chromosome plant will be, we must suppose, to modify the various characters of the plant, and probably to make it more like *vulgare*.

These expectations are borne out by an examination of the F_2. It is clear that some pairs of characters show Mendelian inheritance, though it will be shown later that complications occur; and so we get new combinations of existing characters in the usual way, but this process requires no special comment and will not be considered further. Of the new types that appear in F_2 some no doubt owe their existence to the possession of various numbers and combinations of the 7 extra chromosomes; but these will not breed true, since no plants breed true to an intermediate number of chromosomes: plants with less than 35 chromosomes never give offspring with more chromosomes than they themselves possess, and the number 28 is attained fairly rapidly; while plants with more than 35 chromosomes never give offspring with less chromosomes than they have themselves and the number 42 is reached

sooner or later. It is possible that to this cause must be assigned the fact that so far I have been unable to isolate a fairly common new type that is characterised by having remarkably narrow glumes.

It can be stated as a matter of experience that plants with more than 35 chromosomes resemble *vulgare* rather than *turgidum*; such plants will therefore only give *vulgare*-like plants when bred from, and, with the attainment of 42 chromosomes, pure breeding forms that can be assigned with complete confidence to the species *vulgare* can be obtained. Similarly, plants with less than 35 chromosomes are more like *turgidum*; they give only *turgidum*-like plants, and eventually true breeding 28 chromosome forms, undoubtedly *turgidum* in their characters, are obtained. Finally, any plant with 35 chromosomes behaves cytologically like the F_1 and gives both *vulgare* and *turgidum* types amongst its offspring.

In this description of F_2, and later types, a relation between chromosome number and type—*turgidum* or *vulgare*—has been stated. It is important now to trace more exactly how close this association is. From what was said in describing the species it appears that although the parent forms are very different, and the species to which they belong are well defined, yet there are very few characters that are always found in one species and never found in the other; and if we consider a far larger number of characters than are mentioned here this still holds good, though no doubt the more closely we searched the more we should find. Probably the two most valuable diagnostic characters are the leaf hair arrangement, and the keeled or rounded glume; and of these two, while the former remains associated with chromosome number when the species are crossed, the second does not: we can easily obtain round-glumed, 28-chromosome, *turgidum* types that breed true. Hence, the fact that a character is absolutely confined to one species and not normally found in the other is no guarantee that it cannot be transferred by crossing. Also, we must ask what justification we have in calling an extracted 28-chromosome plant a *turgidum* when it no longer has some of the typical *turgidum* characters. Aside from the leaf hair character which is absolutely associated with chromosome number, as are some other less easily defined characters, the justification for this is that many characters that are associated with the particular varieties used as parents, rather than with the species, may also be associated with chromosome number. For example, though all *turgidums* have a solid straw the character does not absolutely define the species, since in a few *vulgare* forms the straw is solid; but we cannot produce a solid-

strawed *vulgare* by crossing *turgidum* with the hollow-strawed Iron; so in the cross this character has diagnostic value. Since other characters that are not necessarily specific also behave in this way the types we extract from the species cross are not difficult to classify if we examine them critically.

Because of the association with chromosome number, the frequency with which *vulgare*-like and *turgidum*-like types will appear in F_2 clearly depends both upon the frequency with which the unpaired chromosomes are lost during the reduction divisions of the $F_1(5)$, and upon the selective action of sterility on pollen grains with different numbers of chromosomes; to a less extent it may also be influenced by the non-germination of some grains. Probably in all crosses between members of the 28 and 42 chromosome groups there is loss of chromosomes at reduction, and consequently megaspores and microspores with 14, 15 chromosomes are more frequent than those with 21, 20 (for details see earlier papers). For this reason, provided no other irregularity obscures the result, plants with less than 35 chromosomes—*turgidum*-like plants—will outnumber in F_2 the plants with more than 35 chromosomes —*vulgare*-like plants; and the greater the loss of chromosomes at reduction the greater will be the numerical preponderance of the former class. In the Swedish Iron × Rivet cross an excess of *turgidum* types in F_2 occurs in this way; and in other wheat species crosses it is possible that a much heavier loss of chromosomes might entirely prevent the reappearance of the species with the higher chromosome number unless a very large F_2 were grown. But the frequency of the types in F_2 also depends upon the relative efficacy of pollen with high and with low chromosome numbers. In the Swedish Iron × Rivet cross there does not appear to be any marked difference here; but there is evidence that in some crosses the greater fertility of pollen grains with the higher chromosome numbers might cause a preponderance in F_2 of the higher chromosome type of zygote, and of course the opposite might be the case and this type would be still further diminished.

In the above description the simplest possible type of behaviour has been assumed. Exceptions might occur if a *turgidum* chromosome paired with one of the *vulgare* chromosomes that normally remain unpaired, instead of with its usual homologue; other irregularities are also possible.

After this brief account of the species and the nature of the F_2 we shall go on to describe the back-crosses that have been made between the F_1 and the two parent forms, and the bearing of these results on the question of pollen sterility.

GRAIN GERMINATION.

A knowledge of the germination of the grains obtained from the reciprocal back-crosses between F_1 and the parent species is important in connection with the later genetic studies and will be given here in addition to data for other crosses which throw some light on the cause of bad germination.

Many investigators have commented on the poor germination of grains derived from crosses between species of the second (28-chromosome) and third (42-chromosome) wheat groups, but so far as I am aware the fact that reciprocal crosses give markedly different results has not been recorded. No doubt the amount of germination depends to some extent upon conditions, but at Cambridge the experience of several different years shows that, whatever the species employed, the grains given by 42-chromosome ♀ × 28-chromosome ♂ germinate well and those from the reciprocal cross badly. This is illustrated by the following results:

TABLE I.

Cross	Year sown		No. sown	No. germinated
	1923	(1)	10	10
		(2)	23	22
	1924	(1)	43	34
Vulgare ♀ × _turgidum_ ♂		(2)	58	51
	1925	(1)	29	20
		(2)	16	11
	1926		17	15
Vulgare ♀ × _dicoccum_ ♂	1924		21	17
	1924		11	9
Vulgare ♀ × _persicum_ ♂		(1)	6	6
	1926	(2)	22	19
		(3)	10	9
Spelta ♀ × _turgidum_ ♂	1926		11	10
	Total		277	233 = 84 %

TABLE II.

Cross	Year sown		No. sown	No. germinated
		(1)	45	19
	1923	(2)	17	6
Turgidum ♀ × _vulgare_ ♂		(3)	17	7
	1924	(1)	30	10
		(2)	38	12
Dicoccum ♀ × _vulgare_ ♂	1923		10	7
Persicum ♀ × _vulgare_ ♂	1924		23	1
Turgidum ♀ × _Spelta_ ♂	1924		35	16
Durum ♀ × _Spelta_ ♂	1926		14	9
	Total		229	87 = 38 %

In both tables the numbers (1), (2), etc. mean that different varieties of one or the other species were used as parents in the different cases.

The difference between the two series is well marked despite the wide range of species used. Now, according to genetic theory the embryos in reciprocal crosses are identical, and in none of the above cases have I been able to detect any differences between the growing plants. But the endosperms in the two series differ widely: instead of being triploid for all chromosomes, they are diploid or haploid for the extra chromosomes of the 42 species according to whether one of these species is the mother or the father; the actual numbers being 56 and 49 respectively. If we call the extra 7 chromosomes of the 42 group species the 7 *V*-chromosomes, germination is good—but probably not quite perfect—when the 7 *V*-chromosomes are present in the diploid state, and bad when they are present in the haploid state. That the difference in germination is due to the endosperm is confirmed by the fact that the grains from the first series are well filled, though small[1], and differ conspicuously from those of the second series which are always very wrinkled (badly filled).

Now if we press the matter further we find that in the reciprocal back crosses between (*vulgare* × *turgidum*) F_1 and the parent forms we find the same state of affairs: germination is good in the two crosses that give endosperms with the 7 *V*-chromosomes either diploid or triploid, but bad if some of them are only haploid.

TABLE III.

Cross	Grains sown	Grains germinated
Turgidum ♀ × F_1 ♂	37	24
Vulgare ♀ × F_1 ♂	37	35
F_1 ♀ × *turgidum* ♂	42	38
F_1 ♀ × *vulgare* ♂	54	33

The chromosome combinations in the endosperm can readily be worked out. It will be seen, for example, that in the cross F_1 ♀ × *turgidum* ♂ the polar nuclei of the F_1 may contain any number from 0 to 7 of the 7 *V*-chromosomes but, whatever the number, the fusion of the two nuclei renders them diploid; but in F_1 ♀ × *vulgare* ♂ the pollen grain always introduces all the 7 *V*-chromosomes, and since the polar nuclei each may contain only say 1, 2 or 3, etc., of these then 6, 5 or 4, etc., of the 7 *V*-chromosomes will be present in the haploid state in the triple fusion nucleus. The four reciprocal back-crosses therefore fall within the generalisation given above.

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[1] From records made in one year it appears, as would be expected, that similar small grains are obtained from *V* ♀ × F_1 ♂ when 14 chromosome gametes have functioned; 17–21 chromosome gametes give larger, normal sized, grains.

Here again confirmation is supplied by the fact that germination is bad in those crosses that give wrinkled grains. Critical examination suggests that there may be some seasonal variation in amount of wrinkling, but the cross $F_1 \female \times V \male$ always gives wrinkled grains; $T \female \times F_1 \male$, which is considered in detail later, always contains some grains that are very wrinkled; the other two crosses show some variation, but wrinkling is always slight, and in some cases the grain from these crosses compared favourably with well ripened samples from pure lines.

Finally, evidence to the same effect is given by a detailed examination of the cross $T \female \times F_1 \male$. In the year 1925 grains from this cross were classified into wrinkled and not wrinkled and sown separately with the following results:

	Sown	Germinated
Wrinkled	22	11
Not wrinkled	15	13

Though some of the former class were very wrinkled, in others the wrinkling was less marked. Bad germination has affected the wrinkled class almost entirely. Now this class gave 8 plants known to have 31 chromosomes or more, from direct counts; 1 believed to have 31 or more from genetic evidence (see later), and only 1 with 28. The other class, which germinated well, gave 6 plants known to have 28 chromosomes; 2 known to have 28 or 29; 4 believed to have 28 on genetic evidence; and only 1 with 31 chromosomes. Evidently we can classify our grains for number of chromosomes (either 28, or from 31 to 35), almost with certainty, simply by examining them for wrinkling: only two mistakes were made out of the 24 grains that germinated. Furthermore it certainly seems to be grains that possess haploid chromosomes that fail to germinate. It is true that the embryo, as well as the endosperm, have these chromosomes in the haploid condition, but this also applies to most of the embryos from the reciprocal cross—$F_1 \female \times T \male$—and these grains nearly all germinate.

We are forced to conclude that it is the endosperm that is primarily responsible for the bad germination in all these crosses; that failure arises when some chromosomes are present in the endosperm in the haploid condition; and that germination is good if all chromosomes are present in the endosperm in the diploid or triploid condition. In reaching this conclusion we should not be led to think that the constitution of the embryo is without influence on germination: it seems highly probable that success or failure depends on the relations of embryo and

endosperm to each other. If we examine the series of crosses for 28 ♀ × 42 ♂ we find a significant variation from cross to cross; and this, together with other evidence that it is hoped to present in a later paper, suggests that the factorial composition of the embryo may be an important factor.

The F_1 Pollen.

It was shown in the earlier papers that about 20 per cent. of the F_1 pollen grains are completely or partially empty; and that of the total pollen only a low proportion—from 5 to 30 per cent.—germinates on the stigmas. The development of the pollen from the microspores and the connection between chromosome number and pollen sterility will here be described.

Shortly after the reduction divisions are completed the microspores enlarge rapidly and the characteristic pollen grain wall is laid down (Fig. 3). In the grains that develop normally the nucleus then divides, while the cytoplasm and its inclusions are still scanty, and the two daughter nuclei travel to opposite sides of the young pollen grain (Fig. 4). The cytoplasm then begins to increase in volume, but before the pollen grain is completely full the smaller of the two nuclei divides to form the two male gametes (Fig. 5). In the mature pollen grain filling is complete. The development of other microspores is abnormal in that it may cease altogether at any stage after that of Fig. 3 (Figs. 6 and 7), so that a ripe anther besides containing normal well filled pollen grains contains grains that are still at any of the stages from that of Fig. 3 onwards; in addition there are grains that are empty or with their contents disorganised, due to degeneration setting in at any stage of development (Fig. 7). As the pollen germination tests have shown that some apparently perfect grains do not germinate on the stigma (6), while others probably only protrude the tip of the pollen tube, and furthermore other results suggest that the tubes from some grains do not grow very far or grow slowly, it seems that it is possible to trace a complete series between a microspore that ceases development at the stage of Fig. 3 and a pollen grain by means of which fertilisation is accomplished. It has been concluded earlier that this sterility is selective with respect to the number of chromosomes contained, parental numbers functioning most and intermediate numbers more rarely, and this has been confirmed by counting the chromosomes in the plants obtained from crosses between the F_1 pollen and the two parent forms.

Material was provided by 37 grains from the cross Iron ♀ × (Rivet ×

Iron F_1) ♂, and by 37 grains from Rivet ♀ × (Rivet × Iron F_1) ♂. We have seen (p. 384) that in the former cross no error arises from bad grain germination; and from the 35 grains that germinated 31 plants were harvested (one plant died from injury caused by wheat bulb fly, *Hylemyia coarctata*, and in three plants the only ear was fixed for a chromo-
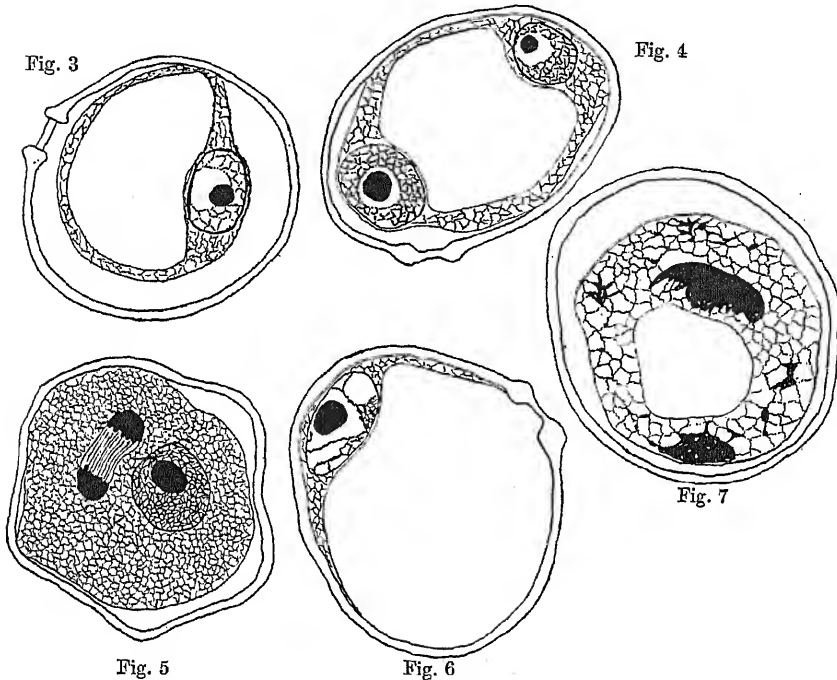
Figs. 3–7. Drawn with the aid of camera lucida. Magnification × 700. Figs. 3–5. Normal development of pollen grain from microspore. Figs. 6 and 7. Abnormal development. The grains shown were from the same anther as the pollen grain of Fig. 5, which represents the stage reached by most of the pollen in the anther. In Fig. 6 development has been arrested at the stage of Fig. 3. In Fig. 7 degeneration has set in after the stage shown in Fig. 4 had been reached. In an ordinary variety of wheat all the pollen grains develop concurrently.

some count) and the chromosomes of 19 were counted. In the second cross it has been shown (p. 385) that bad germination has increased the relative proportion of 28 chromosome plants obtained; 24 plants were obtained from the cross, and the chromosomes of 17 of these were counted. In all cases care was taken to ensure that the plants from which successful counts were obtained represented a random selection from the whole; experience shows that serious error could ensue if this precaution were not taken. The results given for each cross are the sum

of the results obtained in two successive years. Those for F_1 pollen were obtained from Iron × Rivet F_1, and those for F_1 egg-cells from Yeoman × Rivet F_1. Most of the counts are accurate; those that are less certain are given separately and are probably all correct within one.

TABLE IV.

		Frequency of F_1 gametes with the various possible chromosome numbers							
		14	15	16	17	18	19	20	21	Total
Turgidum ♀ × F_1 ♂	Accurate	6	0	0	2	2	1	2	1	14
	Approximate	1	1	0	0	1	0	0	0	3
	Total	7	1	0	2	3	1	2	1	17
Vulgare ♀ × F_1 ♂	Accurate	4	2	0	0	1	1	1	2	11
	Approximate	2	0	0	1	1	0	3	1	8
	Total	6	2	0	1	2	1	4	3	19
Total from two crosses		13	3	0	3	5	2	6	4	36
F_1 ♀ × *turgidum* ♂		2	4	2	1	1	1	0	0	11

The results are enough to show that the effect of sterility is to favour the functioning of pollen grains that have or approach the parental numbers, and to cut out those with intermediate numbers. The expectation for the egg-cell frequency was a binomial distribution with the mode probably at 16 (see earlier papers for a fuller discussion). A discussion of the agreement between the observed and expected frequencies for the egg-cells can best be left until more counts have been made, and for this reason an estimate of the proportion of pollen grains that are eliminated will not yet be given.

Table IV does not show any very definite difference between the frequencies for F_1 pollen functioning on the *turgidum* and the *vulgare* parents respectively; nor does indirect evidence suggest that any decided difference exists. The plants obtained from V ♀ × F_1 ♂ can be classified on genetic evidence (see later, p. 390) into two groups: those with 35 or 36 chromosomes, and those with from 38–42. The value so obtained for the ratio fertilisations by 14 or 15 chromosome pollen : fertilisations by 17–21 chromosome pollen is $11/22 = 0.50$. For the cross T ♀ × F_1 ♂ the same ratio is best found by classifying the grains obtained into wrinkled and not wrinkled (see p. 385) when we have the value $15/22$, a difference that may easily be due to errors of sampling. Some counts given by Kihara[3] suggested that in some crosses a decided difference might exist.

Genetic Aspects of Pollen Sterility.

Direct observations have shown that the development of a pollen grain may be arrested at any stage, and that apparently sound grains may not germinate. There is so far no proof of different rates of growth of pollen tubes, but in view of the many cases where this has now been reported for other plants it seems quite likely that it occurs in these partially sterile wheat hybrids as well. In the future it may be found that no real distinction can be drawn between these phenomena, but whether this be so or not they must be considered here as one since none of the available evidence allows us to discriminate between their effects, and in the subsequent discussion I include these different possibilities when the term sterility is used. A further possibility that should be remembered is selective fertilisation, by which I mean that genetically different egg-cells of one and the same plant might exert a differential attraction on genetically different male gametes. I am not aware that this has ever been proved to occur, and so far I have no evidence pointing directly to its occurring in these crosses, but it is possible that more extensive data might reveal it. It could not of course affect the actual back crosses but would, if it occurred, affect the composition of the following generation.

We have seen that sterility tends to cut out the pollen grains with intermediate chromosome numbers. Since all the F_1 egg-cells are fertile, the genetic effect of this sterility can be ascertained by comparing reciprocal crosses between the F_1 and the parent forms. In making these comparisons no attention will be paid, at present, to characters that clearly depend on a single Mendelian factor and have no special association with either of the parent species; or to certain characters whose inheritance is dealt with elsewhere (7). We shall confine ourselves, instead, principally to some of the characters that are used in diagnosing the species. For the sake of greater simplicity we shall describe only those crosses in which Swedish Iron was the variety of *vulgare* used: the results obtained with Yeoman were similar in principle.

Germination of the grains obtained from the crosses has been given already. By examining the results given by the different crosses it will be seen that the poor germination of grains from $F_1 \, ♀ \times V \, ♂$ and from $T \, ♀ \times F_1 \, ♂$ cannot have affected in any essential way the conclusions reached.

We shall first compare the two crosses $V \, ♀ \times F_1 \, ♂$ and $F_1 \, ♀ \times V \, ♂$. To summarise it may be said that when F_1 is the male parent the plants

obtained fall readily into two groups: plants like the F_1 (F group, 8 plants), and plants like *vulgare* (V group, 22 plants). All plants found to have 35 chromosomes fell into the F group, and all plants with from 38 to 42 chromosomes into the V group; of the two plants with 36 chromosomes only one gave an ear and this will not at present be considered. When, on the other hand, F_1 is the female parent no such clear separation into two groups is possible and the plants (29 in all) tend on the whole to be intermediate between F_1 and *vulgare*. Under each of the headings that follow we have therefore to consider three groups of plants: (1) V group, (2) F group, and (3) $F_1 \female \times V \male$; (1) and (2), of course, both come from the cross $V \female \times F_1 \male$.

(*a*) In vigour, height, and size of ears, the plants from the three groups differ strikingly. Some of the V group plants equal the *vulgare* parent in these respects, and all may be classed as from moderate to good; F group plants are poor; plants from $F_1 \female$ range from poor to moderate.

(*b*) The fertility of the plants was measured by counting the total number of grains set in the lowest two flowers of all the spikelets of a single ear. Thus in an ear with 20 spikelets 40 flowers were examined, and if these gave 20 grains the fertility was 0·50. In each plant one ear had been enclosed in a paper bag to prevent out-pollination, and it was the fertility of this ear that was measured. The same method of measuring fertility was given in an earlier paper[6]. The results were:

	Proportion of grains					Total No. of plants
	0·0–0·2	0·2–0·4	0·4–0·6	0·6–0·8	0·8–1·0	
No. of plants $F_1 \female \times V \male$	4	7	8	6	4	29
„ $V \female \times F_1 \male$	4	5	2	2	13	26*

* Owing to oversight the fertility of four of the V group plants from $V \female \times F_1 \male$ was not measured.

On the whole the plants from the first cross tend to have an intermediate amount of fertility, and those from the second cross either a high or a low fertility. In the latter cross it is the F group plants that are more sterile, and the V group plants that are more fertile:

	Proportion of grains					Total No. of plants
	0·0–0·2	0·2–0·4	0·4–0·6	0·6–0·8	0·8–1·0	
No. of plants F group	3	4	1	0	0	8
„ V „	1	1	1	2	13	18

The difference here is very marked, but a few plants that closely resemble *vulgare* have a low fertility; this will not be discussed further at present.

(c) Plate XXIV, figs. 1 and 2, shows single glumes taken from a representative selection of the plants obtained from the two series of crosses, one glume per plant. The plants selected include all plants harvested in 1925 from $F_1 ♀ × V ♂$ (none of those harvested in 1926); all F group plants, whether harvested in 1925 or in 1926; and most of the V group plants harvested in the two years, those not included being similar to those that are included. Before comparing these glumes it must be explained that glume shape is greatly influenced by the factor K which gives the keel to the glume of *turgidum* ((7), where it is shown that *turgidum* is KK and *vulgare* kk), so in Plate XXIV, the glumes are shown classified for this factor. The effect of K is, besides giving the keel to the glume, to flatten the face of the glume (not readily seen in the Plate), to give it a flatter top or shoulder, to increase its thickness, to increase the development of the lateral nerves, to give a collar at the base (the collar is not shown in the Plate), and to increase the laxity of the ear. If, now, we examine kk glumes from $V ♀ × F_1 ♂$ we find that those of the V group are nearly all typical of *vulgare*, and differ from those of the F group chiefly by being flatter on the face and with a flatter top; while Kk glumes of the two groups differ equally clearly, the most obvious differences being the flatter face, flatter top, and better developed lateral nerves of those of the V group. In fact the difference between the two groups is similar to the change produced by the factor K. Turning now to the glumes from the reciprocal cross, $F_1 ♀ × V ♂$, we find that on the whole they tend to be intermediate between the F group and V group types, and while they resemble *vulgare* on the whole, few, if any, can be regarded as typical of that species.

(d) All F group plants had grey chaff, while all but one of the V group plants had the chaff white or red.

(e) Ear density is greatly influenced by the presence or absence of K, but allowing for this all ears of F group plants are distinctly denser than those of the V group, though the latter show variation in density, and some are fairly dense. Plants from $F_1 ♀ × V ♂$ are more difficult to separate into two groups; most are fairly dense, some as dense as F group plants, and few lax like the V group.

(f) Classification for solid and hollow straw was difficult to make in these crosses, as there is an appreciable amount of fluctuation from tiller to tiller of the same plant, and the F_1 itself, though having a distinct lining of pith, is much closer to *vulgare* than to *turgidum*. It was possible to distinguish, however, a greater amount of pith in the F group as a whole than in the V group. In other straw characters it

was possible to detect more *turgidum* influence in the progeny of $F_1 ♀ × V ♂$ than in V group progeny. In comparing straws of these two groups of plants judgment is influenced by diameter (greater in *vulgare* than in *turgidum*), colour, whether circular (*turgidum*) or elliptical (*vulgare*), and by the texture of the surface, which in its turn is mainly influenced by the degree of development of the longitudinal bands of fibres found in *turgidum* straws and not in *vulgare*. Judgment of straws is not easily carried out on individual plants unless differences are very well marked, which is not the case in the plants under discussion.

(*g*) The progeny of the three groups of plants show well marked differences. F group plants all have 35 chromosomes in those cases where a count has been made. As would be expected therefore they behave like the F_1 in giving very variable progeny, including plants that are mainly *turgidum*-like as well as plants that are more like *vulgare*; the progeny also lack vigour and are highly sterile. The progeny of V group plants, apart from segregation for a few characters such as rough and smooth chaff, are strikingly uniform in ear and in foliage characters, and are very similar to *vulgare*; while many closely resemble the variety of *vulgare* used in making the crosses. The progeny of $F_1 ♀ × V ♂$ were, as a whole, much more variable than the V group, less vigorous, less fertile, and showed stronger traces of *turgidum* in the characters dealt with above.

The reciprocal crosses between *turgidum* and F_1 will now be considered. As before we have three groups of plants to consider: (1) Plants from $T ♀ × F_1 ♂$ that resemble *turgidum* (T group, 13 plants); 8 plants known to have 28 or 29 chromosomes fell into this group. (2) Plants from the same cross in which traces of *vulgare* could be detected ($T+$ group, 10 plants); the nine plants known to have from 31 to 35 chromosomes fell into this group. (3) Plants from $F_1 ♀ × T ♂$.

(*a*) In vigour the plants from $F_1 ♀ × T ♂$ vary from poor to good; few, if any, are as vigorous as the most vigorous plants from the reciprocal cross. In the latter, the T group are all vigorous, though varying rather in amount; many of the plants in the $T+$ group are very lacking in vigour but a few are fairly vigorous.

(*b*) The results for fertility were:

	Proportion of grains					Total No. of plants
	0·0–0·2	0·2–0·4	0·4–0·6	0·6–0·8	0·8–1·0	
No. of plants $F_1 ♀ × T ♂$	11	4	4	6	5	30*
,, $T ♀ × F_1 ♂$	0	4	2	7	10	23

* In a few plants no ear was bagged. The fertility of these is not given here.

On the whole the first cross gives low or intermediate fertility, while F_1 ♂ gives intermediate or high fertility. In this latter cross the plants that resemble *turgidum* (T group) are the more fertile, while the plants of abnormal type or with well marked *vulgare* influence ($T+$ group) are more sterile:

		Proportion of grains					Total No. of plants
		0·0–0·2	0·2–0·4	0·4–0·6	0·6–0·8	0·8–1·0	
No. of plants T	group	0	0	0	3	9	12
,,	$T+$,,	0	4	2	3	1	10

(c) The glumes are shown in Plate XXIV, figs. 4 and 5, and within each group are classified into KK and Kk. Considering the KK series first, it is remarkable that all glumes from T ♀ × F_1 ♂ are very similar to the parental *turgidum* and show little or no trace of *vulgare* influence, with the single exception of the large glume third from the right in the row, which shows this influence strongly in the flat top, well developed
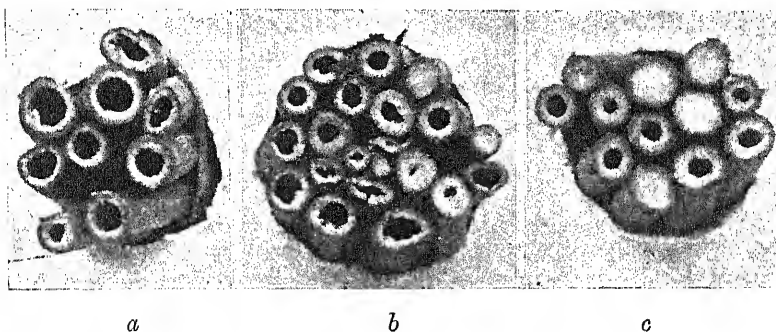
Fig. 8. Straws, cut at 3 inches below the ear to show degree of solidity. *a*, $T+$ group plants from the cross *turgidum* ♀ × F_1 ♂; *c*, T group plants from the same cross; *b*, plants from the cross F_1 ♀ × *turgidum* ♂. One straw per plant. Magnification × ⅓.

lateral nerves, and flat face. In F_1 ♀ × T ♂ at least 6 glumes out of 16 resemble *turgidum* closely, and in the rest it is difficult to see features that can be attributed to *vulgare*. In the Kk series on the other hand it is possible to see *vulgare* influence, chiefly the flatter top, in varying degrees in the $T+$ group, and to a smaller extent in some of the glumes from F_1 ♀ × T ♂.

To sum up, *vulgare* influence appears in the glumes of only one KK plant from either of the reciprocal crosses between *turgidum* and F_1; this needs explanation and will be discussed in a later paper. In Kk plants, however, it still holds good that the cross T ♀ × F_1 ♂ gives two groups of plants—the T group like *turgidum*, and the $T+$ group showing

traces of *vulgare*; while F_1 ♀ × T ♂ gives a number of plants showing *vulgare* traces and less easily separable into two groups.

(*e*) Moderately lax ears are found only among KK plants of the $T+$ group and a few plants from F_1 ♀ that had traces of other *vulgare* characters.

(*f*) Allowing for fluctuation, the straws from T group plants were distinctly more solid than those from the $T+$ group (cf. Fig. 8), and most of the latter approached the F_1 in hollowness. F_1 ♀ × T ♂ gives a few plants with solid straws; most are more or less hollow, but few are as hollow as the F_1. The straws were compared by examining them at 1 inch and at 3 inches below the ear. Rivet is always solid at the first of these points and at the second, if not quite solid, which it usually is, has only a very small lumen.

(*g*) Progeny. The most uniform families came from T group plants, and those from $T+$ group were variable. Some families from F_1 ♀ were variable, but some were fairly uniform,

It was shown earlier in this paper that the functional pollen from *vulgare* × *turgidum* F_1 falls into two classes with regard to chromosome number: pollen with 14 chromosomes (and perhaps a few with 15), and pollen with from 17–21 chromosomes. In the two series of back crosses we have just been studying we have seen that 14 chromosome pollen carries mainly *turgidum* characters, and 17–21 chromosome pollen mainly *vulgare* characters. On the other hand the F_1 egg-cells, which have chiefly intermediate chromosome numbers, tend to be intermediate genetically between *turgidum* and *vulgare*. To some extent this effect may be attributed to the characters that distinguish *vulgare* from *turgidum* being determined by the extra 7 *vulgare* chromosomes, but an alternative possibility must be borne in mind. If a certain *vulgare* character were due to a factor V which is carried by one of the chromosomes that pair with the *turgidum* chromosomes, then V and v would be distributed at random to the gametes; but it might happen that 14 chromosome-v pollen functioned while 14 chromosome-V pollen was eliminated, partially or completely. While this possibility points to the need for caution, I have no doubt that an association between the extra chromosomes and some of the *vulgare* characters does exist.

In studying these crosses attention has been directed to the shape of the glume, the density of the ear, and to certain straw characters, while confirmatory evidence is given by a consideration of vigour and fertility. The number of characters studied was not large, but the general conclusions reached are amply confirmed by a close examination

of the plants themselves, though a closer definition and description of differences is difficult to make. Comment must be made on the frequency with which plants like the parent forms are obtained from the crosses. So far, considering both the crosses in which Yeoman as well as those in which Iron was the *vulgare* parent, 60 plants have been obtained from the cross $F_1 ♀ \times T ♂$; and we have seen that there has been no elimination of types through sterility. Yet out of these 60 plants at least 4 were typically *turgidum* and did not differ much from Rivet. Moreover these 4 plants were homozygous for the factor for keel and for the factor for awns, and only 21 plants in all were homozygous for these factors; and again, some of the plants that were less typically *turgidum* presumably owed this to their possessing some extra *vulgare* chromosomes. In view of the fact that the F_1 has 14 segregating pairs of chromosomes and that $2^{14} =$ about 17,000 this is very surprising; and it would seem that we must regard the *turgidum* chromosomes as very similar to the 14 from *vulgare* with which they pair. I think this must be taken as strong evidence for a simple polyploid relationship between the two species, but even so the closeness of the similarity is surprising, and it is hoped that a critical investigation of this question can be carried out.

SUMMARY.

The paper gives a preliminary account of the genetics of crosses between *Triticum turgidum* with 14 chromosomes (haploid) and *T. vulgare* with 21. The cytology of the cross has been described in earlier papers. It is pointed out that although the two species are sharply separated from one another, and easily recognised, there are few characters that are found in all forms of the one and no forms of the other. Moreover one of the characters that serves absolutely to distinguish the species, and the one usually relied on, is the glume keel found in *turgidum* and not in *vulgare*, and this character can be transferred from one species to the other by crossing. Yet if we extract from the cross forms with 28 and forms with 42 chromosomes the two series so obtained are still sharply separated from one another; partly because some characters that distinguish the parent varieties, rather than the species, are associated with chromosome number.

In crosses between wheat species of the second (28 chromosome) and third (42 chromosome) groups, and in certain back crosses described in the text, grain germination is largely determined by the chromosome constitution of the endosperm. Germination is good if the extra *vulgare*

chromosomes are all diploid or triploid in the endosperm but bad if some of them are only present in the haploid condition.

The development of pollen grains from microspores, in the partially sterile *vulgare* × *turgidum* F_1, has been described.

Reciprocal back crosses between the F_1 and the two parent forms have been studied. Direct counts have confirmed the earlier, indirect conclusion, that the fully fertile F_1 egg-cells mostly have a chromosome number intermediate between 14 and 21; and that in the F_1 pollen the sterility falls most heavily on the pollen grains with intermediate chromosome numbers, and greatly increases the chance that fertilisation will be effected by those with the parental numbers.

Considering some of the characters that differentiate the species, it is found that 14 chromosome pollen carries mainly *turgidum* characters, and 17–21 chromosome pollen mainly *vulgare* characters. The egg-cells tend on the whole to be genetically intermediate.

Comment is made on the fact that the results do not appear to disclose the existence of many factor differences between the 14 *turgidum* chromosomes and the 14 from *vulgare* with which they pair, and that strong evidence for a simple polyploid relationship between the two species is obtained in this way.

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EXPLANATION OF PLATES XXIII—XXIV.

PLATE XXIII.

Fig. 1. Ears of: *a, T. turgidum* var. Rivet; *b, F_1; c, T. vulgare* var. Swedish Iron. Magnification × ½.

PLATE XXIV.

Figs. 1, 2, 4 and 5, one glume per plant from the reciprocal back crosses; classified within each cross for the factor *K*. Fig. 3, two glumes each from *T. turgidum* var. Rivet, F_1 and *T. vulgare* var. Swedish Iron; Fig. 1, *vulgare* ♀ × F_1 ♂; Fig. 2, F_1 ♀ × *vulgare* ♂; Fig. 4, *turgidum* ♀ × F_1 ♂; Fig. 5, F_1 ♀ × *turgidum* ♂. Magnification × ⅔.

F group   *V* group

Kk

kk

Fig. 1. $V\,♀ \times F_1\,♂$

Kk

kk

Fig. 2. $F_1\,♀ \times V\,♂$

turgidum F_1 *vulgare*

Fig. 3.

T group   *T+* group

KK

Kk

Fig. 4. $T\,♀ \times F_1\,♂$

KK

Kk

Fig. 5. $F_1\,♀ \times T\,♂$